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Santos, Raquel ORCID: 0000-0003-3129-6732, Guirguis, Amira and Davidson, Colin ORCID: 0000-0002-8180-7943 (2020) How preclinical studies have influenced novel psychoactive substance legislation in the UK and Europe. British Journal Of Clinical Pharmacology, 86 (3). pp. 452-481. ISSN 0306-5251

It is advisable to refer to the publisher's version if you intend to cite from the work.
http://dx.doi.org/10.1111/bcp.14224

How Pre-Clinical Studies Have Influenced Novel Psychoactive Substance Legislation in The UK and Europe

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Key words: novel psychoactive substance, legal high, legislation, toxicity, abuse

Running Head: review of NPS pharmacology

The authors have no conflicts of interest to declare

Abstract

Novel psychoactive substances (NPS) are new drugs of abuse. Over the last 10 years 50-100 new NPS have been detected for the first time each year. This has led to numerous deaths and challenges to healthcare providers and law-makers worldwide. We review pre-clinical studies of NPS and discuss how these studies have influenced legislative decisions. We focus on the UK legal system but include experiences from Europe. We reviewed manuscripts from 2008-2019 and have summarised the in-vitro and in-vivo data on NPS, highlighting how these studies define pharmacological mechanisms and how they might predict 'harm' in humans. We found that only a small percentage of NPS have been examined in pre-clinical studies. Most pre-clinical studies of NPS focus on basic pharmacological mechanisms (46% of studies reviewed) and/or addictive liability (32%) rather than toxicity and 'harm' (24%). Very few pre-clinical studies into NPS include data from chronic dosing schedules (9%) or female rodents (4%). We conclude that pre-clinical studies can predict harm to humans, but most of the predictions are based on basic pharmacology rather than demonstrated toxicity. Some of these studies have been used to make changes to the law in the UK and Europe and perhaps, because of the paucity of toxicology data, most NPS have been placed in the highly dangerous 'schedule 1' or Class A category in the UK. We suggest that in-silico studies and high throughput toxicology screens might be the most economical way forward to rapidly screen the health harms of NPS.

1. Introduction

Over the last decade, the drug scene has completely changed. In 2018 a 'new' Novel Psychoactive Substance (NPS) was reported to the European Commission Early Warning System every week with 55 NPS reported in total. This is a much lower number than that reported in 2013-2015 (1). Despite the fact that the observed decline could have resulted from many factors such as international legislation or possible "lack of innovation", many substances continued to resurge globally (1), indicating a new established illicit repertoire. Other approaches employed to capture the true number of NPS that are globally available includes using a 24/7 web crawler such as the 'NPS.Finder®' (2). This novel approach showed that the online scenario includes over 4000 unique psychoactive molecules of interest to psychonauts, a number that is approximately four-fold the number being reported to the known NPS databases (European Monitoring Centre on Drugs and Drug Addiction (EMCDDA) and United Nations Office on Drugs and Crimes (UNODC)) (2).

In the UK, NPS were originally known as "*psychoactive drugs which are not prohibited by the United Nations Single Convention on Narcotic Drugs or by the Misuse of Drugs Act 1971, and which people in the UK are seeking for intoxicant use*" (3). They were therefore initially emerging to circumvent international legislation. In the UK, the Psychoactive Substances Act (PSA) 2016 broadly defined them as "*any substance, which is capable of producing a psychoactive effect*" (4), covering any substance that is not controlled by the Misuse of Drugs Act 1971. Under the latter definition, the UK Home Office issued a forensic strategy to test for the psychoactivity of an unknown substance (5), which is key for prosecution in the UK (6). In this respect, in-vitro testing was recommended by the ACMD (Advisory Council on the Misuse of Drugs) to prove if a substance is psychoactive. This mandates positive receptor and functional assays at one or more of the following receptors or transporters: CB_1 (cannabinoid receptor type 1), $GABA_A$ (γ-aminobutyric acid A receptor), $5HT2_A$ (serotonin 2A receptor), NMDA (N-methyl-D-aspartate receptor), μ-opioid receptor and monoamine transporters (dopamine, serotonin, norepinephrine). However, since in-vitro testing may not be suitable for all substances (e.g. nitrous oxide and solvents), in-vivo testing has been recommended as an alternative. In vitro testing can predict human doses for stimulants and psychedelics, for example (7) but cannot easily predict human pharmacokinetics or brain penetration. Information may also be retrieved from scientific literature if in-vitro/ in-vivo testing cannot be performed (5) .

Despite the apparent decline in the numbers of emerging NPS, research is lagging behind the rapid changes in this complex market. Case reports including clinical and forensic cases, and emergency and mental health admissions have shown that NPS are associated with unpredictable acute adverse

events, serious harms and even deaths (8). Knowledge of long-term effects following chronic use of NPS is lacking and systems that capture harms resulting from NPS are still patchy (9).

Animal models have been previously validated and have played pivotal roles in pre-clinical research informing the neurobiological, psychopathological, behavioural and etiological aspects underlying drug addiction and acute/ chronic drug use (10 - 11). They have long been used to inform on the various phases of drug addiction including drug self-administration patterns, behavioural criteria that define vulnerability to drug abuse, conditioned place preference, craving and relapse, escalation of drug-taking, impulsivity, continued drug-taking despite adverse drug reactions, physical dependence and other neuropsychopathological aspects, which lead from a "voluntary" drug-taking to "compulsive" behaviours (10 - 14). Furthermore, Preclinical studies have contributed to understanding the toxicity spectrum associated with some traditional drugs of abuse including knowledge of neurotoxic effects seen with amphetamines (15) and MDMA (16), as well as non-neurological toxic effects such as MDMA-induced heart-valve issues (17) and bladder problems associated with ketamine use (18).

Recent reviews of NPS included studies which focussed on toxicity such as Assi et al., (2017) (19) who reviewed the literature between 2007-2015 for NPS toxicity, yielding 20 studies and concluded that the harmful effects of NPS could be severe or lethal. Tracy et al., 2017 (20) summarised NPS-induced health harms as deduced from available clinical data, while Evans-Brown and Sedefov (2018) reviewed the NPS risk assessment protocols across the EU (21).

Here we review the pre-clinical studies of NPS over the last 10 years and summarise the findings from the main classes of NPS. We then examine the evidence that has been used in changing the legal status of NPS focussing on the UK with some examples from Europe.

2. Materials and Methods

A literature search was performed on PubMed and Web-of-Science (Medline), from January 2008 to November 2019. We employed database-specific search strategies with multiple keywords utilising word truncation/ wild card symbols and index terms as appropriate for each database. The literature search was not undertaken using individually named NPS due to their large number, up to 4000 by some estimates (2); consequently, not all pertinent studies may be identified.

Each identified article was categorised into the following topics (based on title and abstract review): pharmacological and behavioural experiments (preclinical studies), human studies (clinical), detection and identification (forensic), legal status (legal), epidemiology, comments, letters, replies (reports) and reviews.

2.1 Search strategy and study selection NPS

The following search terms were used in PubMed:

(((((((legal high[Title/Abstract]) OR novel psychoactive substance[Title/Abstract]) OR new psychoactive substance[Title/Abstract]) OR bath salt[Title/Abstract]) OR designer drug[Title/Abstract]) OR plant food[Title/Abstract])) AND ((((((((((in-vitro[Title/Abstract]) OR in-vivo[Title/Abstract]) OR cell[Title/Abstract]) OR slice[Title/Abstract]) OR anesthetized[Title/Abstract]) OR anaesthetised[Title/Abstract]) OR rat[Title/Abstract]) OR mouse[Title/Abstract]) OR murine[Title/Abstract]) OR rodent[Title/Abstract]) NOT review

In Web-of-Science, we selected the Medline database with the following search terms:

TI= (legal highs* or novel psychoactive substance* or new psychoactive substance* or bath salt* or designer drug* or plant food*) and MH=(in-vivo* or in-vitro* or cell* or slice* or anesthetised* or rat* or mouse* or rodents*).

The literature search yielded 335 articles from both PubMed and Web-of-Science (Figure 1). Additional 22 articles were found deemed to be relevant. Fourty-seven articles were excluded due to duplicates. Three-hundred-and-ten articles were screened for inclusion, and 66 were excluded they were deemed irrelevant. Two-hundred-and-five articles were screened for eligibility, and 136 were excluded in the final screening because they did not meet the following criteria: 1) NPS was not a major outcome; 2) the article was a clinical study; 3) the article was a forensic study; or 4) the article was a review. In total, 108 journal articles from the original search were used in the final analysis (Figure 1).

<Figure 1 is about here>

3. Summary of results from NPS searches

After excluding ineligible manuscripts, we found 55 animal (in-vitro or in-vivo) studies examining cathinones, 25 studies on stimulants, only eight studies on synthetic cannabinoid receptor agonists (SCRAs) and 20 studies on 'other' NPS. We have grouped the types of studies undertaken into four broad areas: 1) behavioural models of addiction; 2) neurochemistry/ neuropharmacology of addiction; 3) toxicity; and 4) cognitive dysfunction.

3.1. Cathinones

The most commonly studied cathinone was MDPV (3,4-Methylenedioxypyrovalerone) (25 studies (45%)), followed by mephedrone and methylone (16 studies (29%)) and α-PVP (α-pyrrolidinovalerophenone) (7 studies (13%)). All other cathinones studied were only examined in three or fewer manuscripts: (Table 1). Most studies examined these substances in models of addiction

such as increased locomotion, locomotor sensitisation, conditioned place preference, drug self-administration, increased intracranial brain stimulation and withdrawal effects; there were 31 (56%) such studies. The next most common type of study were neurochemical assays associated with dopamine, serotonin or noradrenaline; these mostly examined changes in these transmitter levels or effects on their transporters; there were 17 such studies (31%). The next most common type of study was related to toxicity: cell death, cell toxicity, reactive oxygen species (ROS) or hyperthermia and there were 11 such studies (20%). Three studies (5%) examined cardiovascular issues such as blood pressure or heart rate, one looked at hepatotoxicity, three (5%) looked at cognitive deficits such as memory impairment and one study (2%) examined the effects of cathinones on rodent offspring. Only 2 studies (4%) examined chronic effects of cathinones and only 2 studies (4%) reported using female rodents.

<Table 1 is about here>

3.2. Stimulants.

The most studied NPS stimulants were TFMPP (3-Trifluoromethylphenylpiperazine), N-benzylpiperazine and 4,4'-DMAR (4,4'-Dimethylaminorex), which were all studied in four manuscripts (16%). The other stimulants were only studied in three or less manuscripts (Table 2). The NPS stimulants were mostly studied using neurochemical assays at dopamine, serotonin or noradrenaline systems (16 studies (64%)) with six studies (24%) examining toxicity or hyperthermia, three studies (12%) looked at the behavioural effects using models of addiction and a single study used assays related to the cardiovascular system. Thus, similar to the cathinones, the vast majority of studies examined either behavioural or neurochemical indices of addiction and very few looked at acute toxic effects. No studies examined chronic effects of stimulants and no studies reported using female rodents.

<Table 2 is about here>

3.3. SCRAs

There have been very few pre-clinical studies on SCRAs over the last 10 years with only 10 or so SCRAs examined in eight studies. These include JWH- and CP-compounds while AKB48 was the most studied SCRA (3 studies (38%)). Despite the lack of studies, it is clear that the types of assay differ from both the cathinone and stimulant research. In the SCRA manuscripts, five studies (63%) examined cell toxicity, five studies (63%) examined their neuropharmacology, one study looked at cardiovascular effects and one study looked at models of addiction. Thus, in contrast to the cathinone and stimulant

studies, there was only one study looking models of addiction and proportionally more studies examined toxicity (Table 3).

We note previous studies on SCRAs from outside our search period (Jan 2008 - Dec 2018), for example Randall et al., (2004) (22). In this article, we focussed only on studies from the last 10 years, which include the so-called third generation SCRAs (23 - 24).

<Table 3 is about here>

3.4 Other NPS

There are relatively few studies on 'other' NPS (20 studies) with the most studied drugs being methoxetamine and 25B-NBOMe (both 4 studies, 20%). Most of the assays used are to examine the neuropharmacology of the drugs (12 studies (60%)) with only one manuscript examining effects of the drugs in models of abuse. Four studies (20%) looked at the toxicity of these drugs, two studies (10%) found cognitive dysfunction, one found bladder and renal toxicity and one looked at cardiovascular effects (Table 4).

<Table 4 is about here>

3.6. Overview of pre-clinical data on NPS (more formal and supported)

Taken together we can see that most recent studies on NPS examined the reinforcing effects or addictive liability of NPS, whether through behavioural models (32%) or neurotransmitter changes such as dopamine levels (46%). Taking NPS for their rewarding effects is one of the motivations for use, other reasons for using NPS including self-medicating to treat withdrawal symptoms from other drugs or the availability when traditional drugs are scarce or supplementing the illicit drug use by trying to induce synergistic or additive effects. From a legislative point of view, it might be useful to know if a drug was more or less addictive and this criterion is used by the EU for banning NPS (25). Further, more resources, whether it be policing, research, healthcare or education could be targeted at those NPS predicted to be more addictive. The next most common type of study examined cell toxicity (24%). This is useful as highly toxic substances should clearly be banned and this criterion is also used by the EU. Interestingly, the effects of these drugs, which might result in acute hospitalisation, for example cardiotoxicity, seizure activity, hyperthermia, or death, have hardly been studied at all in NPS (all <5%).

Only 4% of studies included female rodent samples and only 9% included chronic dosing studies. The age range of most studies was appropriate with most (72%) using rodents, which were adolescent or in early adulthood (100 – 299 g) and only 22% of studies examining older rodents (> 300 g).

4. Predictive validity of NPS tests in animal tissue

As detailed above, when NPS started flooding the market over the last decade or so, pre-clinical tests were exploited to compare these substances to their traditional counterpart, describing them as "cocaine-like" or "amphetamine-like" substances(26 - 27). Unlike known illicit substances such as methamphetamine and MDMA (3,4-methylenedioxymethamphetamine), the pharmacology related to emerging NPS is scarce and may not suitably be extrapolated from existing knowledge. For example, when the risk assessment of 2C-I (2,5-dimethoxy-4-iodophenethylamine) was carried out in 2003, a "speculative comparison" was made with the phenethylamine analogue 2C-B (2,5-dimethoxy-4-bromophenethylamine) and the amphetamine analogue DOB (2,5-dimethoxy-4-bromoamphetamine), as they both have similar chemical structures to 2C-I but with a bromine instead of iodine. It was deemed inappropriate to compare or extrapolate data related to the structurally similar MDMA, PMA (*paramethoxyamphetamine*) and 4-MTA (4-methylthioamphetamine) due to the absence of the 2,5-dimethoxy functional group (28).

The validity of animal models in understanding the harmful effects of illicit substances in humans is well established and therefore, can provide a predictive validity for NPS-related harms. For instance, ketamine has been associated with bladder toxicity (29). Similar to ketamine, animal models showed significant bladder and renal toxicity in rodents following the administration of 30 mg/kg methoxetamine intraperitoneally (30). Symptoms included "inflammatory cell infiltration, tubular cell necrosis, glomerular damage" and "increased micturition frequency bladder dysfunction"(30) (31).

Benzofurans such as 5-APB (5-(2-aminopropyl)benzofuran), 5-APDB (5-(2-aminopropyl)-2,3-dihydrobenzofuran), 6-APB (6-(2-aminopropyl)benzofuran), 6-APDB (5-(2-aminopropyl)-2,3-dihydrobenzofuran), 4-APB (4-(2-aminopropyl)benzofuran), 7-APB (7-(2-aminopropyl)benzofuran), 5-EAPB (5-(2-ethylaminopropyl)benzofuran) and 5-MAPDB (1-(2,3-dihydrobenzofuran-5-yl)-*N*-methylpropan-2-amine) are all structurally similar to MDMA (32). Similar to MDMA, they were found to activate the 5-HT_{2B} receptor (Dawson et al., 2014), which induces heart valve fibrosis (33 - 35) and inhibit dopamine transporters (DAT) (36 - 37).

MDPV (3,4-methylanedioxypyrovalerone) is a cathinone derivative with a nitrogen atom in the pyrrolidine ring and a 3,4-methylenedioxy group on the phenyl ring similar to MDMA (38). In-vitro and in-vivo rodent models showed that MDPV blocks the dopamine and norepinephrine transporters in a similar way to the pyrovalerone analogues. It is more potent at both the dopamine and norepinephrine transporters and less potent blocking serotonin in a similar way to cocaine (38 - 39). The potent blockade of dopamine and epinephrine stipulates that MDPV has the potential of inducing a high risk of abuse/ addiction, and life-threatening cardiovascular stimulation including tachycardia

and hypertension respectively, more than cocaine (38 - 39). This was consistent with in-vitro data assessing blood-brain barrier permeability, in-vivo microdialysis and in-vivo locomotor activity testing in rats (26, 38 – 39).

One problem with pre-clinical studies is whether or not the toxic dose or concentration from the pre-clinical study reflects clinical doses. There is a wealth of literature on MDMA toxicity in the 5-HT system, but this has been criticised because the doses needed to show neurotoxicity in animals may be far above the clinically relevant doses (40).

Taken together, we can see that pre-clinical studies can be used to predict health harms. Some researchers have gone a step further, using in-silico studies to predict addictive liability of NPS. The benzofurans 5-APB and 5-MAPB were predicted to bind to DAT in a similar way to MDMA (37), while ketamine-like NPS dipehidine and methoxphenidine were predicted to bind differently at DAT (41).

5. Evidence used in UK legislation and in Europe

5.1. Evidence used in the UK

In the UK, the government takes advice on issues around drugs of abuse from the ACMD who risk assess emerging substances and issue subsequent recommendations (42). The ACMD also advises the government on the control of drugs and drugs requiring a temporary class drug order. In their first NPS report, on BZP (benzylpiperazine), they recommended that the drug and some of its 1-phenyl and 1-benzyl derivatives be brought under the Misuse of Drugs Act (1971): "*The ACMD considers that the harms and misuse of BZP and substituted piperazines (identified in Annex 4) are commensurate with Class C, under schedule 2, part III, of the Misuse of Drugs Act (1971); and should be scheduled under Schedule I of the Misuse of Drugs Regulations (2001) (having no recognised medicinal use).*" This decision was based on a report by the EMCDDA (43). In this report there is evidence from pre-clinical studies in the 1970s and 80s that BZP had addictive liability and had similar properties to amphetamine or MDMA. These studies were undertaken as the drug was being examined for antidepressant effects. More recent data from the 2000s is focused on human use and in particular BZP use in New Zealand where it was a popular party drug. This data suggest that the drug should not be used by people susceptible to seizures or in those with cardiac conditions. It is difficult to attribute blame to a particular drug in many of the human studies as in most cases the drug takers, although being shown to have BZP in their systems, may have consumed numerous other substances. Thus, although the UK government banned BZP, as it was obliged to do by the European Commission, the direct evidence for toxicity was limited because much of the

data came from polydrug users. Nevertheless, its known pharmacological profile and its suspected effects on humans clearly merited some sort of control.

In another early publication, this time 'advice' to the then Home Secretary Theresa May, on D2PM (Ivory wave) which "*…typically cause prolonged agitation (lasting up to 5 days after drug use which is sometimes severe, requiring physical restraint), paranoia, hallucinations and myoclonus (muscle spasms/twitches)*", the ACMD recommended that there was an immediate ban on the import of 2-DPMP. This 'advice' was followed a year later by a 'report' on desoxypipradrol (3), the ACMD could again rely on data from previous studies as desoxypipradrol had been tested for narcolepsy. After considering information from hospital emergency rooms, published research, drug company data and coroners reports the advice was to bring desoxypipradrol and its associated drugs D2PM and 2-diphenylmethylpyrrolidine under the Misuse of Drugs Act (1971). The clinical evidence used to ban desoxypipradrol included three studies: a case report of a man who had taken D2PM with agitation and chest pains (44); five case reports have shown the presence of D2PM rather than desoxypipradrol and the victims exhibited signs of agitation, anxiety and insomnia but not any sympathomimetic toxicity (45); two case reports involving polydrug abusers, again presented with agitation, anxiety and insomnia but without increased heart rate, hypertension or hyperthermia (46). None of these studies actually confirmed desoxypipradrol use. Recent preclinical data has shown that desoxypipradrol was more potent than cocaine at inhibiting dopamine reuptake (47). Perhaps the best evidence to ban desoxypipradrol came from a report by Ciba-Geigy in the 1950s showing that desoxypipradrol tended to have a much lower LD_{50} (lethal dose, 50%) in animals than amphetamine or methamphetamine.

The ACMD went on to provide a number of reports and advice on various NPS including cathinones (2010), methoxetamine (2012), benzofurans (2013), N-BOMe, AH-7921 (3,4-dochloro-N-[[1-(dimethylamino)cyclohexyl]-benzamide), tryptamines, MT-45 (1-cyclohexyl-4-(1,2-diphenylethyl)-piperazine) and 4,4'DMAR (all 2014), MPA (methiopropamine) (2016), third generation SCRAs (2016), methylphenidate-like NPS (2017) and 2-4-dinitrophenol (2019). Not all NPS had a previous history as a potential medicine, where there were previous pre-clinical and clinical studies on the compound. For example, see the EMCDDA risk assessments where the pharmacodynamic profiles of emerging substances such as SCRAs on other pharmacological targets other than CB_1 is very limited. This is particularly true for AB-CHMINACA (N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide), ADB-CHMINACA (N-

(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide), 5F-MDMB-PINACA (5F-ADB or methyl-2-{[1-(5-fluoropentyl)-1H-indazole3-carbonyl]amino}-3,3-dimethylbutanoate), CUMYL-4CN-BINACA (1-(4-cyanobutyl)-N-(2-phenylpropan-2-yl)indazole-3-carboxamide) and many more .

In May 2016, the UK government adopted a different approach aimed at limiting the supply of NPS and capturing substances that escaped the Misuse of Drugs Act 1971 (4) rather than on evidence-based risk assessments. However, as explained above, implementation of the Act is highly reliant on in-vitro databases of a representative set of molecules (6). This is quite limited given the time taken to develop these libraries and the limitations of these libraries when facing emerging previously-unseen substances.

Take together we can see that pre-clinical evidence has previously played an important role in deciding to ban NPS in the UK and continues to support the implementation of the new legislation. The clinical evidence, mostly case reports, is somewhat unreliable due to polydrug use and co-morbities, amongst other issues. Much of the preclinical evidence only suggested potential harms, rather than showing actual toxicity, and is dogged by the obvious need to extrapolate from animals to humans.

5.2. Overview of evidence used in Europe

In Europe, the process has been quite similar to that described above for the UK. The EMCDDA have published numerous reports and advice on NPS. These started with BZP (2007, 2009), Spice (2009), 4-MA (2012), MDPV, methoxetamine, AH7921, 25I-NBOMe, 4,4'-DMAR and MT-45 (2014) and more recently a number of reports on SCRAs and synthetic opioids. In their report (25) on "*New Psychoactive Substances in Europe; Legislation and Prosecution; Current challenges and Solutions*" they describe four broad attempts of member countries controlling NPS. First, by trying to control NPS under laws around medicinal products, but this was thrown out by the Centre for Justice for the European Union on the grounds that NPS were not medicines. Second, some countries have tried to use existing laws around existing consumer safety laws. Third, existing drug laws have been modified by using group definitions of some NPS. Fourth, new laws have been developed, for example the UK PSA 2016 described above. In these new laws the criteria used to define a psychoactive substance is often different and most use an element around harm or threat to health (including dependence) and a criterion that the drug has a psychoactive effect. Clearly, without pre-clinical data, and preferably human data, it is difficult to say with any certainty that an NPS has a psychoactive effect or could cause harm. Some countries also define

psychoactivity quite clearly in their legislation with Ireland requiring 'significant' mental disturbance or change and Hungary and Portugal requiring the NPS to have "*effects similar to established drugs of abuse and a likelihood to cause dependence*" (25).

The EMCDDA have published 22 risk assessments for NPS. The most recent assessment will be used as an example. This was a risk assessment of the synthetic opioid cyclopropylfentanyl. The EMCDDA and Europol examined the available information based upon the following criteria: *"(1) the amount of the material seized; (2) evidence of organised crime involvement; (3) evidence of international trafficking; (4) analogy with better-studied compounds; (5) evidence of the potential for further (rapid) spread; and (6) evidence of cases of serious intoxication or fatalities*." They reported a 'pharmacological description' of a few studies mostly limited to examining the effects of this NPS at μ-opioid receptors in-vitro where it was shown to be more potent than morphine or fentanyl (48) and a single animal study, in mice, suggesting analgesic properties (49). The health risks were suggested to come from accidental overdose and no acute or chronic toxicity studies had been carried out, nor had its dependence liability been examined. It was assumed that this NPS would have toxicity similar to morphine or fentanyl. The report described deaths associated with cyclopropylfentanyl, which included 78 in Sweden, three in the UK, one in Norway and over 100 in the USA (48), albeit in nearly all cases users had recently used multiple drugs. The report was submitted to the European Commission and Council of the European Union, who decided that cyclopropylfentanyl should be subject to control measures across member states. Thus, the EU banned this NPS based on very little direct pharmacological or toxicology data and include information on criminality as a criterion to ban the substance.

6. Conclusions

Clearly it would be beneficial to know as much as possible about each NPS, however scientific curiosity needs to be tempered by the reality of the current drug scene where the vast majority of problems are caused by relatively few established drugs of abuse. It is possible that some NPS may join these established drugs of abuse as major players in the morbidity and mortality associated with recreational drug use and it is these most commonly used NPS that we should focus upon. However, horizon scanning for the next MCAT/mephedrone or Spice/SCRA is also important. We have summarised the pre-clinical data on NPS above and given examples of these data being used in UK and EU legislation. It is reasonable to ask if the changes in legislation have been evidence-based? Nutt and colleagues have published in this area for established drugs of abuse and concluded that UK and Australian scheduling of drugs of abuse is not particularly evidence-based. They highlight the easy

access to nicotine and alcohol, despite these drugs being ranked as highly dangerous. On the other hand, Ecstasy, LSD and magic mushrooms, despite being ranked very lowly on the danger scale, are both schedule 1, class A drugs in the UK (50). It is difficult to say that the UK blanket ban (4) on psychoactive substances is evidenced-based. The vast majority of NPS have not been examined at all while those that have been examined, as described above, tend to be looked at for their addictive effects rather than toxic effects. In addition, studies examining the long-term effects of NPS are very rare and these types of studies would be best placed from which to extrapolate health harms. Nevertheless, the ban appears to be having some of the desired effects as recent evidence suggests fewer problems associated with NPS (5). The ACMD and EMCDDA reports are generally evidence-based but limited to a relatively small number of NPS or families of NPS and a relatively small number of supporting studies. Having said that, given the very large number of NPS (up to 4000 by some estimations (2), there is little chance of all of these drugs being looked at in detail. The best that we can realistically hope for is putting many of these drugs through some high throughput screens for receptor/ transporter binding (51 - 52) and high throughput toxicity assays (53). An alternative approach is in-silico testing i.e. modelling the effects of the drugs at receptors or transporters of interest. We have done this for some ketamine-like NPS including diphenidine and methoxphenidine and MDMA and some similar NPS including 5-APB and 5-MAPB (37, 41). We also suggest that future studies focus more on measures of toxicity (e.g. neurotoxicity, vasoconstriction, hepatotoxicity, seizures and hyperthermia) as this has clearly been overlooked with most studies focussing on rodent behavioural or neurochemical (dopamine) measures of dependence. Finally, there are very few pre-clinical studies using tissues from female rodents. This is important as drug effects do show sexual differentiation (54). Thus, in order to get an accurate overview of NPS pharmacology, more studies using female tissue are needed.

7. References

1. EMCDDA. European Drug Report. 2019.
2. Schifano F, Napoletano F, Chiappini S, Guirguis A, Corkery JM, Bonaccorso S, et al. New/emerging psychoactive substances and associated psychopathological consequences. Psychol Med. 2019 Jul;1–13.
3. ACMD (2011) Novel Psychoactive Substances report 2011. https://www.gov.uk/government/publications/novel-psychoactive-substances-report-2011
4. PSA 2016 Psychoactive Substances Act 2016 http://www.legislation.gov.uk/ukpga/2016/2/contents/enacted
5. Home Office. Drugs and Alcohol Unit/Centre for Applied Science and Technology. 2016. Psychoactive Substances Act 2016 Forensic Strategy. Home Office: UK.
6. CPS 2018 Crown Prosecution Service (2018). https://www.cps.gov.uk/legal-guidance/psychoactive-substances
7. Luethi D, Hoener MC, Liechti ME. Effects of the new psychoactive substances diclofensine, diphenidine, and methoxphenidine on monoaminergic systems. Eur J Pharmacol. 2018 Jan;819:242–7.
8. Schifano F, Orsolini L, Duccio Papanti G, Corkery JM. Novel psychoactive substances of interest for psychiatry. World Psychiatry. 2015 Feb;14(1):15–26.
9. Sumnall HR, Evans-Brown M, McVeigh J. Social, policy, and public health perspectives on new psychoactive substances. Drug Test Anal. 2011;3(7–8):515–23.
10. Belin-Rauscent, A. and Belin D. Animal models of drug addiction. Addictions-From Pathophysiology to Treatment. 2012. 1–30 p.
11. Gurney SMR, Scott KS, Kacinko SL, Presley BC, Logan BK. Pharmacology, Toxicology, and Adverse Effects of Synthetic Cannabinoid Drugs. Forensic Sci Rev. 2014 Jan;26(1):53–78.
12. Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL. Animal models of substance abuse and addiction: implications for science, animal welfare, and society. Comp Med. 2010 Jun;60(3):177–88.
13. Nutt D, Mclellan AT. Improve Addiction Treatment and Policies ? 2013;35(2):1–12.
14. Shippenberg, T.S. and Koob, G.F. (2002). Recent advances in animal models of drug addiction and alcoholism. *Neuropsychopharmacology: The fifth generation of progress*, *13*, pp.81-97.
15. Davidson C, Gow AJ, Lee TH, Ellinwood EH. Methamphetamine neurotoxicity: necrotic and apoptotic mechanisms and relevance to human abuse and treatment. Brain Res Brain Res Rev. 2001 Aug;36(1):1–22.
16. Halpin LE, Collins SA, Yamamoto BK. Neurotoxicity of methamphetamine and 3,4-methylenedioxymethamphetamine. Life Sci. 2014 Feb;97(1):37–44.
17. Cosyns B, Droogmans S, Rosenhek R, Lancellotti P. Drug-induced valvular heart disease.

Heart. 2013 Jan;99(1):7–12.

18. Myers FAJ, Bluth MH, Cheung WW. Ketamine: A Cause of Urinary Tract Dysfunction. Clin Lab Med. 2016 Dec;36(4):721–44.

19. Assi S, Gulyamova N, Ibrahim K, Kneller P, Osselton D. Profile, effects, and toxicity of novel psychoactive substances: A systematic review of quantitative studies. Hum Psychopharmacol. 2017 May;32(3).

20. Tracy DK, Wood DM, Baumeister D. Novel psychoactive substances: types, mechanisms of action, and effects. BMJ. 2017 Jan;356:i6848.

21. Evans-Brown M, Sedefov R. Responding to New Psychoactive Substances in the European Union: Early Warning, Risk Assessment, and Control Measures. Handb Exp Pharmacol. 2018;252:3–49.

22. Randall MD, Kendall DA, O'Sullivan S. The complexities of the cardiovascular actions of cannabinoids. Br J Pharmacol. 2004 May;142(1):20–6.

23. C Davidson, J Opacka-Juffry, A Arevalo-Martin, D Garcia-Ovejero, E Molina-Holgado, F Molina-Holgado (2017) *Advances in Pharmacology*, Chapter Four - Spicing Up Pharmacology: A Review of Synthetic Cannabinoids From Structure to Adverse Events. 80, 135-168.

24. Zanda MT, Fattore L. Old and new synthetic cannabinoids: lessons from animal models. Drug Metab Rev. 2018 Feb;50(1):54–64.

25. EMCDDA. New psychoactive substances in Europe. 2016.

26. Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu L-H, Huwyler J, et al. Pharmacological characterization of designer cathinones in vitro. Br J Pharmacol. 2013 Jan;168(2):458–70.

27. Opacka-Juffry J, Pinnell T, Patel N, Bevan M, Meintel M, Davidson C. Stimulant mechanisms of cathinones - effects of mephedrone and other cathinones on basal and electrically evoked dopamine efflux in rat accumbens brain slices. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Oct;54:122–30.

28. EMCDDA. Report on the risk assessment of 2C-I, 2C-T-2 and 2C-T-7 in the framework of the joint action on new synthetic drugs. 2004.

29. Chu PS, Kwok SC, Lam KM, Chu TY, Chan SW, Man CW, Ma WK, Chui KL, Yiu MK, Chan YC, Tse ML, Lau FL. 'Street ketamine'-associated bladder dysfunction: a report of ten cases. Hong Kong Med J. 2007 13(4):311-3.

30. Dargan PI, Tang HC, Liang W, Wood DM, Yew DT. Three months of methoxetamine administration is associated with significant bladder and renal toxicity in mice. Clin Toxicol (Phila). 2014 Mar;52(3):176–80.

31. Wang Q, Wu Q, Wang J, Chen Y, Zhang G, Chen J, et al. Ketamine Analog Methoxetamine Induced Inflammation and Dysfunction of Bladder in Rats. Int J Mol Sci. 2017 Jan;18(1).

32. Greene, S.L. Benzofurans and benzodifurans.(2013) In: Dargan, P.I, Wood, D.M., editors. Novel Psychoactive Substances. Amsterdam: Elsevier. pp. 283–392.

33. Bhattacharyya S, Schapira AH, Mikhailidis DP, Davar J. Drug-induced fibrotic valvular heart disease. Lancet (London, England). 2009 Aug;374(9689):577–85.

34. Rickli A, Kopf S, Hoener MC, Liechti ME. Pharmacological profile of novel psychoactive benzofurans. Br J Pharmacol. 2015 Jul;172(13):3412–25.

35. Setola V, Hufeisen SJ, Grande-Allen KJ, Vesely I, Glennon RA, Blough B, et al. 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") induces fenfluramine-like proliferative actions on human cardiac valvular interstitial cells in vitro. Mol Pharmacol. 2003 Jun;63(6):1223–9.

36. Dawson P, Opacka-Juffry J, Moffatt JD, Daniju Y, Dutta N, Ramsey J, et al. The effects of benzofury (5-APB) on the dopamine transporter and 5-HT2-dependent vasoconstriction in the rat. Prog Neuropsychopharmacol Biol Psychiatry. 2014 Jan;48:57–63.

37. Sahai MA, Davidson C, Khelashvili G, Barrese V, Dutta N, Weinstein H, et al. Combined in vitro and in silico approaches to the assessment of stimulant properties of novel psychoactive substances - The case of the benzofuran 5-MAPB. Prog Neuropsychopharmacol Biol Psychiatry. 2017 Apr;75:1–9.

38. EMCDDA–Europol (2013) EMCDDA–Europol 2012 Annual Report on the implementation of Council Decision 2005/387/JHA (New drugs in Europe, 2012), Implementation reports. http://www.emcdda.europa.eu/system/files/publications/819/TDAS14001ENN_466653.pdf

39. Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, et al. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive "bath salts" products. Neuropsychopharmacology [Internet]. 2013;38(4):552–62. Available from: http://dx.doi.org/10.1038/npp.2012.204.

40. Baumann MH, Wang X, Rothman RB. 3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings. Psychopharmacology (Berl). 2007 Jan;189(4):407–24.

41. Sahai MA, Davidson C, Dutta N, Opacka-Juffry J. Mechanistic Insights into the Stimulant Properties of Novel Psychoactive Substances (NPS) and Their Discrimination by the Dopamine Transporter-In Silico and In Vitro Exploration of Dissociative Diarylethylamines. Brain Sci. 2018 Apr;8(4).

42. ACMD: https://www.gov.uk/search/policy-papers-and-consultations?organisations%5B%5D=advisory-council-on-the-misuse-of-drugs&parent=advisory-council-on-the-misuse-of-drugs

43. EMCDDA (2007) Report on the risk assessment of BZP in the framework of the Council decision on new psychoactive substances http://www.emcdda.europa.eu/system/files/publications/560/EMCDDA_risk_assessment_8_132618.pdf

44. Lidder S, Dargan P, Sexton M, Button J, Ramsey J, Holt D, et al. Cardiovascular toxicity associated with recreational use of diphenylprolinol (diphenyl-2-pyrrolidinemethanol [D2PM]). J Med Toxicol. 2008 Sep;4(3):167–9.

45. Wood DM, Puchnarewicz M, Johnston A, Dargan PI. A case series of individuals with analytically confirmed acute diphenyl-2-pyrrolidinemethanol (D2PM) toxicity. Eur J Clin Pharmacol. 2012 Apr;68(4):349–53.

46. Wood, D.M., Puchnarewicz, M., Holt, D.W., Ramsey, J., Dargan, P.I. (2011); Detection of the precursor benzophenone in individuals who have used legal highs containing diphenyl-2-pyyrrolidinemethanol (D2PM). Basic & Clinical Pharmacology & Toxicology; 109 (Suppl. 1): 86. https://www.researchgate.net/publication/285178895_Detection_of_the_precursor_benzophenone_in_individuals_who_have_used_legal_highs_containing_diphenyl-2-pyrrolidinemethanol_D2PM

47. Davidson C, Ramsey J. Desoxypipradrol is more potent than cocaine on evoked dopamine efflux in the nucleus accumbens. J Psychopharmacol. 2011 Jul;26(7):1036–41.

48. United States Drug Enforcement Administration (US DEA) (2017), Cyclopropyl fentanyl. Background information and evaluation of 'three factor analysis' (factors 4, 5, and 6) for temporary scheduling. Drug and Chemical Evaluation Section, Diversion Control Division, Drug Enforcement Administration, Washington, DC. October 2017. Available at: https://www.regulations.gov/document?D=DEA-2017-0013-0003

49. Janssen, P.A.J. and Van der Eycken, C.A.M. (1968), 'The chemical anatomy of potent morphine-like analgesics'. In A Burger (Ed.), Drugs affecting the central nervous system, Marcel Dekker, Inc., New York, Vol. 2, pp. 25-60.

50. Nutt DJ, King LA, Phillips LD. Drug harms in the UK: a multicriteria decision analysis. Lancet (London, England). 2010 Nov;376(9752):1558–65.

51. Iversen L, Gibbons S, Treble R, Setola V, Huang X-P, Roth BL. Neurochemical profiles of some novel psychoactive substances. Eur J Pharmacol. 2013 Jan;700(1–3):147–51.

52. Hondebrink L, Kasteel EEJ, Tukker AM, Wijnolts FMJ, Verboven AHA, Westerink RHS. Neuropharmacological characterization of the new psychoactive substance methoxetamine. Neuropharmacology [Internet]. 2017;123(2017):1–9. Available from: https://doi.org/10.1016/j.neuropharm.2017.04.035

53. Araujo AM, Valente MJ, Carvalho M, Dias da Silva D, Gaspar H, Carvalho F, et al. Raising awareness of new psychoactive substances: chemical analysis and in vitro toxicity screening of "legal high" packages containing synthetic cathinones. Arch Toxicol. 2015 May;89(5):757–71.

54. Becker JB. Sex differences in addiction. Dialogues Clin Neurosci. 2016 Dec;18(4):395–402.

55. Dias D, Ferreira B, Roque R, Rita B, Tomás R, Almeida D De. The new psychoactive substance 3 - methylmethcathinone (3 - MMC or metaphedrone) induces oxidative stress , apoptosis , and autophagy in primary rat hepatocytes at human - relevant concentrations. Arch Toxicol [Internet]. 2019;93(9):2617–34. Available from: https://doi.org/10.1007/s00204-019-02539-x

56. Gannon BM, Mesmin MP, Sulima A, Rice KC, Collins GT. Behavioral economic analysis of the reinforcing effects of "bath salts" mixtures: studies with MDPV, methylone, and caffeine in male Sprague-Dawley rats. Psychopharmacology (Berl). 2019;236(3):1031–41.

57. Luethi D, Kolaczynska KE, Walter M, Suzuki M, Rice KC, Blough BE, et al. Metabolites of the ring-substituted stimulants MDMA , methylone and MDPV differentially affect human monoaminergic systems. 2019;

58. Blough BE, Decker AM, Landavazo A, Namjoshi OA, Partilla JS, Baumann MH, et al. The dopamine, serotonin and norepinephrine releasing activities of a series of methcathinone analogs in male rat brain synaptosomes. Psychopharmacology (Berl). 2019;236(3):915–24.

59. Zhou X, Luethi D, Sanvee GM, Bouitbir J, Liechti ME, Krähenbühl S. Molecular Toxicological Mechanisms of Synthetic Cathinones on C2C12 Myoblasts. 2019;

60. Gerecsei LI, Balazsa T, Echevarria D, Adam A, Zachar G, Csillag A. Selective neuronal death following exposure to methylenedioxypyrovalerone is accompanied by an inhibition of NMDA receptor NR2B subunit expression. Acta Neurobiol Exp (Wars). 2019;79(1):92–100.

61. Kolesnikova TO, Khatsko SL, Eltsov OS, Shevyrin VA, Kalueff A V. When fish take a bath: Psychopharmacological characterization of the effects of a synthetic cathinone bath salt

"flakka" on adult zebrafish. Neurotoxicol Teratol [Internet]. 2019/02/20. 2019;73:15–21. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30796953

62. Mayer FP, Cintulova D, Pittrich DA, Wimmer L, Luethi D, Holy M, et al. Stereochemistry of phase-1 metabolites of mephedrone determines their effectiveness as releasers at the serotonin transporter. Neuropharmacology. 2019 Apr;148:199–209.

63. Eshleman AJ, Nagarajan S, Wolfrum KM, Reed JF, Swanson TL, Nilsen A, et al. Structure-activity relationships of bath salt components: substituted cathinones and benzofurans at biogenic amine transporters. Psychopharmacology (Berl). 2019;236(3):939–52.

64. Yoon HS, Ku MJ, Cai WT, Kim J-H. A novel synthetic cathinone, α-pyrrolidinopentiothiophenone (PVT), produces locomotor sensitization in rat: Implications for GSK3β connections in the nucleus accumbens core. Neurochem Int [Internet]. 2018/12/18. 2019 Mar;124:25–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30571998

65. Atehortua-Martinez LA, Masniere C, Campolongo P, Chasseigneaux S, Callebert J, Zwergel C, et al. Acute and chronic neurobehavioral effects of the designer drug and bath salt constituent 3,4-methylenedioxypyrovalerone in the rat. J Psychopharmacol. 2019;33(3):392–405.

66. Colon-Perez LM, Pino JA, Saha K, Pompilus M, Kaplitz S, Choudhury N, et al. Functional connectivity, behavioral and dopaminergic alterations 24 hours following acute exposure to synthetic bath salt drug methylenedioxypyrovalerone. Neuropharmacology. 2018;137:178–93.

67. Oliver CF, Simmons SJ, Nayak SU, Smith GR, Reitz AB, Rawls SM. Chemokines and 'bath salts': CXCR4 receptor antagonist reduces rewarding and locomotor-stimulant effects of the designer cathinone MDPV in rats. Drug Alcohol Depend [Internet]. 2018;186(January):75–9. Available from: https://doi.org/10.1016/j.drugalcdep.2018.01.013

68. Luethi D, Kolaczynska KE, Docci L, Krahenbuhl S, Hoener MC, Liechti ME. Pharmacological profile of mephedrone analogs and related new psychoactive substances. Neuropharmacology. 2018 May;134(Pt A):4–12.

69. Siedlecka-Kroplewska K, Wrońska A, Stasiłojć G, Kmieć Z. The Designer Drug 3-Fluoromethcathinone Induces Oxidative Stress and Activates Autophagy in HT22 Neuronal Cells. Neurotox Res. 2018;34(3):388–400.

70. Zwartsen A, Hondebrink L, Westerink RH. Neurotoxicity screening of new psychoactive substances (NPS): Effects on neuronal activity in rat cortical cultures using microelectrode arrays (MEA). Neurotoxicology [Internet]. 2018;66:87–97. Available from: https://doi.org/10.1016/j.neuro.2018.03.007

71. Gerecsei LI, Csillag A, Zachar G, Gévai L, Simon L, Dobolyi Á, et al. Gestational exposure to the synthetic cathinone methylenedioxypyrovalerone results in reduced maternal care and behavioral alterations in mouse pups. Front Neurosci. 2018;12(FEB):1–14.

72. Gannon B.M., Rice K.C. CGT. Reinforcing effects of abused'bath salts'constituents3,4-methylenedioxypyrovalerone andα-pyrrolidinopentiophenone and their enantiomers. Behav Pharmacol. 2017;28(7):578–81.

73. Philogene-Khalid HL, Simmons SJ, Nayak S, Martorana RM, Su SH, Caro Y, et al. Stereoselective Differences between the Reinforcing and Motivational Effects of Cathinone-Derived 4-Methylmethcathinone (Mephedrone) In Self-Administering Rats. ACS Chem Neurosci. 2017 Dec;8(12):2648–54.

74. Nelson KH, Hempel BJ, Clasen MM, Rice KC, Riley AL. Conditioned taste avoidance, conditioned place preference and hyperthermia induced by the second generation 'bath salt' α-pyrrolidinopentiophenone (α-PVP). Vol. 156, Pharmacology Biochemistry and Behavior. 2017. p. 48–55.

75. Gannon BM, Russell LN, Modi MS, Rice KC, Fantegrossi WE. Effects of orally self-administered bath salt constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice. Drug Alcohol Depend [Internet]. 2017;179(June):408–15. Available from: http://dx.doi.org/10.1016/j.drugalcdep.2017.06.031

76. Lantz SM, Rosas-Hernandez H, Cuevas E, Robinson B, Rice KC, Fantegrossi WE, et al. Monoaminergic toxicity induced by cathinone phthalimide: An in vitro study. Neurosci Lett [Internet]. 2017;655:76–81. Available from: http://dx.doi.org/10.1016/j.neulet.2017.06.059

77. Luethi D, Liechti ME, Krahenbuhl S. Mechanisms of hepatocellular toxicity associated with new psychoactive synthetic cathinones. Toxicology. 2017 Jul;387:57–66.

78. Grecco GG, Kisor DF, Magura JS, Sprague JE. Impact of common clandestine structural modifications on synthetic cathinone "bath salt" pharmacokinetics. Toxicol Appl Pharmacol. 2017;328:18–24.

79. Elmore JS, Dillon-Carter O, Partilla JS, Ellefsen KN, Concheiro M, Suzuki M, et al. Pharmacokinetic Profiles and Pharmacodynamic Effects for Methylone and Its Metabolites in Rats. Neuropsychopharmacology [Internet]. 2017;42(3):649–60. Available from: http://dx.doi.org/10.1038/npp.2016.213

80. McLaughlin G, Morris N, Kavanagh P V., Power JD, Dowling G, Twamley B, et al. Synthesis, characterization and monoamine transporter activity of the new psychoactive substance mexedrone and its N-methoxy positional isomer, N-methoxymephedrone. Drug Test Anal. 2017;9(3):358–68.

81. Schindler CW, Thorndike EB, Suzuki M, Rice KC, Baumann MH. Pharmacological mechanisms underlying the cardiovascular effects of the "bath salt" constituent 3,4-methylenedioxypyrovalerone (MDPV). Br J Pharmacol. 2016;173(24):3492–501.

82. Berquist MD 2nd, Traxler HK, Mahler AM, Baker LE. Sensitization to the locomotor stimulant effects of "bath salt" constituents, 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (MDPV), in male Sprague-Dawley rats. Drug Alcohol Depend. 2016 Jul;164:128–34.

83. Schindler CW, Thorndike EB, Goldberg SR, Lehner KR, Cozzi N V, Brandt SD, et al. Reinforcing and neurochemical effects of the "bath salts" constituents 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-N-methylcathinone (methylone) in male rats. Psychopharmacology (Berl). 2016 May;233(10):1981–90.

84. Valente MJ, Araújo AM, Bastos M de L, Fernandes E, Carvalho F, Guedes de Pinho P, et al. Characterization of hepatotoxicity mechanisms triggered by designer cathinone drugs (β-keto amphetamines). Toxicol Sci. 2016;153(1):89–102.

85. Colon-Perez LM, Tran K, Thompson K, Pace MC, Blum K, Goldberger BA, et al. The psychoactive designer drug and bath salt constituent MDPV causes widespread disruption of brain functional connectivity. Neuropsychopharmacology [Internet]. 2016;41(9):2352–65. Available from: http://dx.doi.org/10.1038/npp.2016.40

86. Gannon BM, Williamson A, Suzuki M, Rice KC, Fantegrossi WE. Stereoselective effects of abused "bath salt" constituent 3,4-methylenedioxypyrovalerone in mice: Drug discrimination,

locomotor activity, and thermoregulation. J Pharmacol Exp Ther. 2016;356(3):615–23.

87. Lopez-Arnau R, Martinez-Clemente J, Pubill D, Escubedo E, Camarasa J. Serotonergic impairment and memory deficits in adolescent rats after binge exposure of methylone. J Psychopharmacol. 2014 Nov;28(11):1053–63.

88. Kiyatkin EA, Kim AH, Wakabayashi KT, Baumann MH, Shaham Y. Effects of Social Interaction and Warm Ambient Temperature on Brain Hyperthermia Induced by the Designer Drugs Methylone and MDPV. Neuropsychopharmacology [Internet]. 2015;40(2):436–45. Available from: http://dx.doi.org/10.1038/npp.2014.191

89. Hutchinson C V., Prados J, Davidson C. Persistent conditioned place preference to cocaine and withdrawal hypo-locomotion to mephedrone in the flatworm planaria. Neurosci Lett [Internet]. 2015;593:19–23. Available from: http://dx.doi.org/10.1016/j.neulet.2015.03.021

90. Lopez-Arnau R, Martinez-Clemente J, Carbo M li, Pubill D, Escubedo E, Camarasa J. An integrated pharmacokinetic and pharmacodynamic study of a new drug of abuse, methylone, a synthetic cathinone sold as “bath salts”. Prog Neuropsychopharmacol Biol Psychiatry. 2013 Aug;45:64–72.

91. Adam A, Gerecsei LI, Lepesi N, Csillag A. Apoptotic effects of the “designer drug” methylenedioxypyrovalerone (MDPV) on the neonatal mouse brain. Neurotoxicology. 2014 Sep;44:231–6.

92. Den Hollander B, Rozov S, Linden AM, Uusi-Oukari M, Ojanperä I, Korpi ER. Long-term cognitive and neurochemical effects of “bath salt” designer drugs methylone and mephedrone. Pharmacol Biochem Behav [Internet]. 2013;103(3):501–9. Available from: http://dx.doi.org/10.1016/j.pbb.2012.10.006

93. Simmler LD, Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of a new series of designer cathinones. Neuropharmacology [Internet]. 2013/11/22. 2014 Apr;79:152–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24275046

94. Kaizaki A, Tanaka S, Numazawa S. New recreational drug 1-phenyl-2-(1-pyrrolidinyl)-1-pentanone (alpha-PVP) activates central nervous system via dopaminergic neuron. J Toxicol Sci. 2014 Feb;39(1):1–6.

95. Julie A. Marusich, Kateland R. Antonazzo, Jenny L. Wiley, Bruce E. Blough, John S. Partilla and MHB. Pharmacology of novel synthetic stimulants structurally related to the “bath salts” constituent 3,4-methylenedioxypyrovalerone (MDPV). Neuropharmacology. 2014;87:206–13.

96. Watterson LR, Burrows BT, Hernandez RD, Moore KN, Grabenauer M, Marusich JA, et al. Effects of alpha-pyrrolidinopentiophenone and 4-methyl-N-ethylcathinone, two synthetic cathinones commonly found in second-generation “bath salts,” on intracranial self-stimulation thresholds in rats. Int J Neuropsychopharmacol. 2014 Oct;18(1).

97. Bonano JS, Glennon RA, De Felice LJ, Banks ML, Negus SS. Abuse-related and abuse-limiting effects of methcathinone and the synthetic “bath salts” cathinone analogs methylenedioxypyrovalerone (MDPV), methylone and mephedrone on intracranial self-stimulation in rats. Psychopharmacology (Berl). 2014 Jan;231(1):199–207.

98. Gregg RA, Tallarida CS, Reitz AB, Rawls SM. Mephedrone interactions with cocaine: prior exposure to the “bath salt” constituent enhances cocaine-induced locomotor activation in rats. Behav Pharmacol. 2013 Dec;24(8):684–8.

99. Gregg RA, Tallarida CS, Reitz A, McCurdy C, Rawls SM. Mephedrone (4-

methylmethcathinone), a principal constituent of psychoactive bath salts, produces behavioral sensitization in rats. Drug Alcohol Depend. 2013 Dec;133(2):746–50.

100. Gatch MB, Taylor CM, Forster MJ. Locomotor stimulant and discriminative stimulus effects of "bath salt" cathinones. Behav Pharmacol. 2013;24(5–6):437–47.

101. Cameron K, Kolanos R, Vekariya R, De Felice L, Glennon RA. Mephedrone and methylenedioxypyrovalerone (MDPV), major constituents of "bath salts," produce opposite effects at the human dopamine transporter. Psychopharmacology (Berl). 2013 Jun;227(3):493–9.

102. Baumann MH, Jr MAA, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. The Designer Methcathinone Analogs , Mephedrone and Methylone , are Substrates for Monoamine Transporters in Brain Tissue. Neuropsychopharmacology [Internet]. 2011;37(5):1192–203. Available from: http://dx.doi.org/10.1038/npp.2011.304

103. 10.1016/j.pbb.2012.10.006 Den Hollander, 2013

104. López-Arnau R, Martínez-Clemente J, Rodrigo T, Pubill D, Camarasa J, Escubedo E. Neuronal changes and oxidative stress in adolescent rats after repeated exposure to mephedrone. Toxicol Appl Pharmacol [Internet]. 2015;286(1):27–35. Available from: http://dx.doi.org/10.1016/j.taap.2015.03.015

105. Cozzi, N.V., Brandt, S.D., Daley, P.F., Partilla JS, Rothman, R.B., Tulzer, A., Sitte, H.H. and Baumann MH. Pharmacological examination of trifluoromethyl ring-substituted methcathinone analogs. Eur J Pharmacol. 2014;699(0):180–7.

106. Lisek R, Xu W, Yuvasheva E, Chiu Y-T, Reitz AB, Liu-Chen L-Y, et al. Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. Drug Alcohol Depend. 2012 Nov;126(1–2):257–62.

107. Meng H, Cao J, Kang J, Ying X, Ji J, Reynolds W, et al. Mephedrone, a new designer drug of abuse, produces acute hemodynamic effects in the rat. Toxicol Lett. 2012 Jan;208(1):62–8.

108. Baumann MH, Jr MAA, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al. The Designer Methcathinone Analogs , Mephedrone and Methylone , are Substrates for Monoamine Transporters in Brain Tissue. Neuropsychopharmacology [Internet]. 2011;37(5):1192–203. Available from: http://dx.doi.org/10.1038/npp.2011.304

109. Rickli A, Kolaczynska K, Hoener MC, Liechti ME. Pharmacological characterization of the aminorex analogs 4-MAR, 4,4'-DMAR, and 3,4-DMAR. Neurotoxicology. 2019 May;72:95–100.

110. Cai WT, Yoon HS, Lee S, Kim J-H. Repeated exposure to methiopropamine increases dendritic spine density in the rat nucleus accumbens core. Neurochem Int [Internet]. 2019/06/07. 2019 Oct;129:104487. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31176680

111. Maier J, Mayer FP, Luethi D, Holy M, Jäntsch K, Reither H, et al. The psychostimulant (±)-cis-4,4'-dimethylaminorex (4,4'-DMAR) interacts with human plasmalemmal and vesicular monoamine transporters. Neuropharmacology. 2018;138:282–91.

112. Davidson C, Raby CAR, Barrese V, Ramsey J. In vitro neurochemical assessment of methylphenidate and its "legal high" analogs 3,4-CTMP and ethylphenidate in rat nucleus accumbens and bed nucleus of the stria terminalis. Front Psychiatry. 2018;9(MAY):1–9.

113. Luethi D, Kaeser PJ, Brandt SD, Krahenbuhl S, Hoener MC, Liechti ME. Pharmacological profile of methylphenidate-based designer drugs. Neuropharmacology. 2018 May;134(Pt A):133–40.

114. McLaughlin G, Baumann MH, Kavanagh P V., Morris N, Power JD, Dowling G, et al. Synthesis, analytical characterization, and monoamine transporter activity of the new psychoactive substance 4-methylphenmetrazine (4-MPM), with differentiation from its ortho- and meta-positional isomers. Drug Test Anal. 2018;10(9):1404–16.

115. Zwartsen A, Verboven AHA, van Kleef RGDM, Wijnolts FMJ, Westerink RHS, Hondebrink L. Measuring inhibition of monoamine reuptake transporters by new psychoactive substances (NPS) in real-time using a high-throughput, fluorescence-based assay. Toxicol Vitr [Internet]. 2017;45(January):60–71. Available from: http://dx.doi.org/10.1016/j.tiv.2017.05.010

116. Mayer FP, Burchardt N V., Decker AM, Partilla JS, Li Y, McLaughlin G, et al. Fluorinated phenmetrazine "legal highs" act as substrates for high-affinity monoamine transporters of the SLC6 family. Neuropharmacology [Internet]. 2018;134:149–57. Available from: https://doi.org/10.1016/j.neuropharm.2017.10.006

117. Shimshoni JA, Winkler I, Edery N, Golan E, van Wettum R, Nutt D. Toxicological evaluation of 5-methoxy-2-aminoindane (MEAI): Binge mitigating agent in development. Toxicol Appl Pharmacol [Internet]. 2017/02/04. 2017 Mar 15;319:59–68. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28167221

118. McLaughlin G, Morris N, Kavanagh P V., Power JD, Dowling G, Twamley B, et al. Analytical characterization and pharmacological evaluation of the new psychoactive substance 4-fluoromethylphenidate (4F-MPH) and differentiation between the (±)-threo and (±)-erythro diastereomers. Drug Test Anal. 2017;9(3):347–57.

119. Yoon HS, Cai WT, Lee YH, Park KT, Lee YS, Kim JH. The expression of methiopropamine-induced locomotor sensitization requires dopamine D2, but not D1, receptor activation in the rat. Behav Brain Res [Internet]. 2016;311:403–7. Available from: http://dx.doi.org/10.1016/j.bbr.2016.05.060

120. Marusich JA, Antonazzo KR, Blough BE, Brandt SD, Kavanagh P V, Partilla JS, et al. The new psychoactive substances 5-(2-aminopropyl)indole (5-IT) and 6-(2-aminopropyl)indole (6-IT) interact with monoamine transporters in brain tissue. Neuropharmacology. 2016 Feb;101:68–75.

121. Simmler LD, Buchy D, Chaboz S, Hoener MC, Liechti ME. In Vitro Characterization of Psychoactive Substances at Rat , Mouse , and Human Trace Amine-Associated Receptor 1 s. 2016;134–44.

122. Asaoka N, Kawai H, Nishitani N, Kinoshita H, Shibui N, Nagayasu K, et al. A new designer drug 5F-ADB activates midbrain dopaminergic neurons but not serotonergic neurons. J Toxicol Sci. 2016;41(6):813–6.

123. Persona K, Polus A, Góralska J, Gruca A, Dembińska-Kieć A, Piekoszewski W. An In Vitro Study of the Neurotoxic Effects of N-Benzylpiperazine: A Designer Drug of Abuse. Neurotox Res. 2016;29(4):558–68.

124. Arbo MD, Melega S, Stöber R, Schug M, Rempel E, Rahnenführer J, et al. Hepatotoxicity of piperazine designer drugs: up-regulation of key enzymes of cholesterol and lipid biosynthesis. Arch Toxicol. 2016;90(12):3045–60.

125. Arbo MD, Silva R, Barbosa DJ, da Silva DD, Silva SP, Teixeira JP, et al. In vitro neurotoxicity evaluation of piperazine designer drugs in differentiated human neuroblastoma SH-SY5Y cells. Vol. 36, Journal of Applied Toxicology. 2016. p. 121–30.

126. Rubio M, López-Arnau R, Pubill D, Escubedo E, Camarasa J. Serotonin is involved in the

psychostimulant and hypothermic effect of 4-methylamphetamine in rats. Neurosci Lett [Internet]. 2015;590:68–73. Available from: http://dx.doi.org/10.1016/j.neulet.2015.01.075

127. Rickli A, Hoener MC, Liechti ME. Monoamine transporter and receptor interaction profiles of novel psychoactive substances: para-halogenated amphetamines and pyrovalerone cathinones. Eur Neuropsychopharmacol. 2015 Mar;25(3):365–76.

128. McLaughlin G, Morris N, Kavanagh P V, Power JD, Twamley B, O'Brien J, et al. Synthesis, characterization, and monoamine transporter activity of the new psychoactive substance 3',4'-methylenedioxy-4-methylaminorex (MDMAR). Drug Test Anal. 2015 Jul;7(7):555–64.

129. Arbo MD, Silva R, Barbosa DJ, da Silva DD, Rossato LG, Bastos M de L, et al. Piperazine designer drugs induce toxicity in cardiomyoblast h9c2 cells through mitochondrial impairment. Toxicol Lett [Internet]. 2014;229(1):178–89. Available from: http://dx.doi.org/10.1016/j.toxlet.2014.06.031

130. Brandt SD, Baumann MH, Partilla JS, Kavanagh P V, Power JD, Talbot B, et al. Characterization of a novel and potentially lethal designer drug (+/-)-cis-para-methyl-4-methylaminorex (4,4'-DMAR, or 'Serotoni'). Drug Test Anal. 2014;6(7–8):684–95.

131. Bilel S, Tirri M, Arfè R, Stopponi S, Soverchia L, Ciccocioppo R, et al. Pharmacological and Behavioral Effects of the Synthetic Cannabinoid AKB48 in Rats. Front Neurosci [Internet]. 2019 Oct 30;13:1163. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31736697

132. Banister SD, Adams A, Kevin RC, Macdonald C, Glass M, Boyd R, et al. Synthesis and pharmacology of new psychoactive substance 5F-CUMYL-P7AICA, a scaffold- hopping analog of synthetic cannabinoid receptor agonists 5F-CUMYL-PICA and 5F-CUMYL-PINACA. Vol. 11, Drug Testing and Analysis. 2019. p. 279–91.

133. Kim S, Choi W, Kwon M, Lee S, Cho Y, Lee JY, et al. In Vitro Inhibitory E ects of APINACA on Human Major Cytochrome P450, UDP-Glucuronosyltransferase Enzymes, and Drug Transporters. Molecules. 2019;24(December 2016).

134. Kevin RC. CUMYL-4CN-BINACA Is an Efficacious and Potent Pro- Convulsant Synthetic Cannabinoid Receptor Agonist. 2019;10(May):1–9.

135. Domoto M, Sasase H, Wada S, Ito S, Deyama S, Hinoi E, et al. The synthetic cannabinoid 5F-AMB changes the balance between excitation and inhibition of layer V pyramidal neurons in the mouse medial prefrontal cortex. Psychopharmacology (Berl). 2018;235(8):2367–76.

136. De Luca MA, Castelli MP, Loi B, Porcu A, Martorelli M, Miliano C, et al. Native CB1 receptor affinity, intrinsic activity and accumbens shell dopamine stimulant properties of third generation SPICE/K2 cannabinoids: BB-22, 5F-PB-22, 5F-AKB-48 and STS-135. Neuropharmacology. 2016 Jun;105:630–8.

137. Yun J, Yoon KS, Lee TH, Lee H, Gu SM, Song YJ, et al. Synthetic cannabinoid, JWH-030, induces QT prolongation through hERG channel inhibition. Toxicol Res (Camb). 2016;5(6):1663–71.

138. Tomiyama K, Funada M. Cytotoxicity of synthetic cannabinoids on primary neuronal cells of the forebrain: the involvement of cannabinoid CB1 receptors and apoptotic cell death. Toxicol Appl Pharmacol. 2014 Jan;274(1):17–23.

139. Luethi D, Widmer R, Trachsel D, Hoener MC, Liechti ME. Monoamine receptor interaction profiles of 4-aryl-substituted 2,5-dimethoxyphenethylamines (2C-BI derivatives). Eur J Pharmacol. 2019 Jul;855:103–11.

140. Wallach J, Colestock T, Agramunt J, Claydon MDB, Dybek M, Filemban N, et al.

Pharmacological characterizations of the “legal high” fluorolintane and isomers. Eur J Pharmacol [Internet]. 2019/05/29. 2019 Aug 15;857:172427. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31152702

141. Costa G, Serra M, Pintori N, Casu MA, Zanda MT, Murtas D, et al. The novel psychoactive substance methoxetamine induces persistent behavioral abnormalities and neurotoxicity in rats. Neuropharmacology [Internet]. 2018/10/23. 2019 Jan;144:219–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30366005

142. Yoon KS, Gu SM, Lamichhane S, Han KM, Shin J, Kim YH, et al. Methoxetamine Induces Cytotoxicity in H9c2 Cells: Possible Role of p21 Protein (Cdc42/Rac)-Activated Kinase 1. Cardiovasc Toxicol [Internet]. 2019;19(3):229–36. Available from: http://dx.doi.org/10.1007/s12012-018-9489-4

143. Halberstadt AL, Chatha M, Stratford A, Grill M, Brandt SD. Comparison of the behavioral responses induced by phenylalkylamine hallucinogens and their tetrahydrobenzodifuran (“FLY”) and benzodifuran (“DragonFLY”) analogs. Neuropharmacology [Internet]. 2018/10/29. 2019 Jan;144:368–76. Available from: https://www.ncbi.nlm.nih.gov/pubmed/30385253

144. Herian M, Wojtas A, Kamińska K, Świt P, Wach A, Gołembiowska K. Hallucinogen-Like Action of the Novel Designer Drug 25I-NBOMe and Its Effect on Cortical Neurotransmitters in Rats. Neurotox Res. 2019;36(1):91–100.

145. Shintani-Ishida K, Saka K, Nakamura M, Yoshida KI, Ikegaya H. Experimental Study on the Postmortem Redistribution of the Substituted Phenethylamine, 25B-NBOMe. Vol. 63, Journal of Forensic Sciences. 2018. p. 588–91.

146. Cha HJ, Jeon SY, Jang HJ, Shin J, Kim YH, Suh SK. Rewarding and reinforcing effects of 4-chloro-2,5-dimethoxyamphetamine and AH-7921 in rodents. Neurosci Lett [Internet]. 2018;676(April):66–70. Available from: https://doi.org/10.1016/j.neulet.2018.04.009

147. Kang H, Park P, Bortolotto ZA, Brandt SD, Colestock T, Wallach J, et al. Ephenidine: A new psychoactive agent with ketamine-like NMDA receptor antagonist properties. Neuropharmacology [Internet]. 2017;112:144–9. Available from: http://dx.doi.org/10.1016/j.neuropharm.2016.08.004

148. Rickli A, Moning OD, Hoener MC, Liechti ME. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. Eur Neuropsychopharmacol [Internet]. 2016/05/20. 2016 Aug;26(8):1327–37. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27216487

149. Wallach J, Kang H, Colestock T, Morris H, Bortolotto ZA, Collingridge GL, et al. Pharmacological Investigations of the Dissociative “Legal Highs” Diphenidine, Methoxphenidine and Analogues. PLoS One. 2016;11(6):1–17.

150. Blough BE, Landavazo A, Decker AM, Partilla JS, Baumann MH, Rothman RB. Interaction of psychoactive tryptamines with biogenic amine transporters and serotonin receptor subtypes. Psychopharmacology (Berl). 2014 Oct;231(21):4135–44.

151. Paulke A, Kremer C, Wunder C, Achenbach J, Djahanschiri B, Elias A, et al. Argyreia nervosa (Burm. f.): receptor profiling of lysergic acid amide and other potential psychedelic LSD-like compounds by computational and binding assay approaches. J Ethnopharmacol. 2013 Jul;148(2):492–7.

152. Compton DM, Dietrich KL, Selinger MC, Testa EK. 5-methoxy-N,N-di(iso)propyltryptamine hydrochloride (Foxy)-induced cognitive deficits in rat after exposure in adolescence. Physiol

Behav. 2011 May;103(2):203–9.

Table 1. Cathinones.

Study	DOI	NPS	Dose/conc	Species /sex	Age/ weight	Samples	Assay	Main results
Dias da Silva, D. et al., 2019 (55)	10.1007/s00204-019-02539-x	Metaphedrone	31 nM to 10 mM 1 µM,10 µM, 100 µM and 500 µM	Male Wistar Han rats	150 – 250 g	Hepatocytes	Cytotoxic assays	↑ autophagic and apoptotic/necrotic mechanisms
Gannon, B.M. et al., 2019 (56)	10.1007/s00213-018-5046-x	MDPV and methylone	0.32 mg/kg/inf	Male Sprague-Dawley rats	275 –300 g		Self-administration	Methylone + caffeine results in an enhanced reinforcing effectiveness compared to methylone alone
Luethi, D. et al., 2019 (57)	10.1177/0269881119844185	Methylone and MDPV				Human embryonic kidney 293 cells	Monoamine transporter assays	↑ potency to inhibit NA uptake with Methylone compared with DA and 5-HT uptake. ↓ transporter inhibition Potencies with N-demethylation of methylone. = NA and DA uptake inhibition potencies with O-demethylenation of methylone and MDPV.
Blough, B.E. et al., 2019 (58)	10.1007/s00213-018-5063-9	Methcathinone analogs		Male Sprague-Dawley rats	300 - 400 g	Synaptosomes		Compounds substituted at the 2-position (ortho) were primarily dopaminergic Compounds substituted at the 4-position (para) were found to be more serotonergic
Zhou, X. et al., 2019 (59)	10.3390/ijms20071561	3-methylmethcathinone, mephedrone,				C2C12 myoblasts	Cytotoxic assays	All cathinones showed cell membrane integrity, depleted ATP levels, and increased mitochondrial superoxide concentrations

		methylone, MDPV, α-PVP, and naphyrone						α-PVP and naphyrone impaired basal and maximal cellular respiration
Gerecsei, L.I. et al., 2019 (60)		MDPV		Mice		Neural tube	Cytotoxic assays Western blotting	↑ apoptotic cells in the pallium and in the subpallium ↓NR2B expression in the treated group
Kolesnikova, T.O. et al., 2019 (61)	10.1016/j.ntt.2019.02.001	α-PVP	1, 5, 25 and 50 mg/L for 20 min 1, 5 and 10 mg/L for 7 days	Zebrafish			Drug exposure HPLC	α-PVP psychostimulant effects at 5, 25 and 50 mg/L Hypolocomotion and repeated withdrawal after a 7-day chronic treatment
Mayer, F.P. et al., 2019 (62)	10.1016/j.neuropharm.2018.12.032	Enantiomers of nor-mephedrone, 4-hydroxytolyl-mephedrone (4-OH-mephedrone) and dihydro-mephedrone				Human urine	Monoamine transporter assays	↑ potency at SERT with S-enantiomers of nor-mephedrone and 4-OH-mephedrone than the corresponding R-enantiomers. ↓ effectivity as releaser at SERT with R-enantiomers.
Eshleman, A.J. et al., 2019 (63)	10.1007/s00213-018-5059-5	22 cathinones, 6 benzofurans and 1 stimulant				Human embryonic kidney 293 cells	Monoamine transporter assays	Most α-pyrrolidinophenones had high hDAT potencies and selectivities 4-Cl-ethcathinone and 3,4-methylenedioxy-N-propylcathinone had higher hSERT selectivity Benzofurans had low hDAT selectivity and were releasers at hSERT
Yoon, H.S. et al., 2019 (64)	10.1016/j.neuint.2018.12.005	α-PVT	20 mg/kg (i.p)	Rats		Nucleus accumbens	Behavioural experiments	↑ locomotor sensitization ↓ phosphorylation levels of GSK3β

							Western blotting	
Atehortua-Martinez, L.A. et al., 2019 (65)	10.1177/0269881118822151	MDPV	3 mg/kg (i.p)	Male Sprague-Dawley rats	8 weeks 250 – 300 g	Nucleus accumbens, caudate putamen and prefrontal cortex.	Behavioural experiments Western blotting	↑ stereotypies and open arm entries in the elevated plus maze with acute administration ↑ ΔFosB with chronic administration
Colon-Perez, L.M. et al., 2018 (66)	10.1016/j.neuropharm.2018.04.031	MDPV	1 and 3mg/kg (i.p)	Male Long Evans rats	250 - 300 g	Whole brain striatum	Functional magnetic resonance imaging Immunoblotting Dopamine transporter (DAT) assay Social interaction test Ultrasonic vocalization Intracranial self-stimulation	↓ strength of correlated neural activity at 1 h ↓ striatal DAT at 24 h and caused a shift in subcellular levels and distribution of DAT, TH and VMAT2 ↓ ICSS thresholds at 1 h ↑ 50 kHz USV calls at 1 h ↓ social interactions

Oliver, C.F. et al., 2018 (67)	10.1016/j.drugalcdep.2018.01.013	MDPV	2 mg/kg (i.p)	Male Sprague-Dawley rats	275 – 300 g		Locomotor activity CPP Ultrasonic vocalization	AMD3100 reduced MDPV-induced locomotor activation, conditioned place preference and modulated MDPV-induced increase at 50 k-Hz USV calls.
Luethi, D. et al., 2018 (68)	10.1016/j.neuropharm.2017.07.026	5-IT, 4-MA, 3-MMC, N-methyl-2-AI and MMAI				Human embryonic kidney 293 cells	Monoamine transporter assays	4-MA, and MMAI entactogenic effects and 3-MMC, 5-IT, and N-methyl-2-AI have stimulant-type properties
Siedlecka-Kroplewska, K. et al., 2018 (69)	10.1007/s12640-018-9898-y	3-Fluoromethcathinone	10µM	Mice		Hippocampal HT22 cell line	Flow cytometry Western blotting	↑ intracellular production of reactive oxygen species ↑ formation of autophagic vacuoles ↓ level of p62/SQSTM1 protein
Zwartsen, A. et al., 2018 (70)	10.1016/j.neuro.2018.03.007	PMMA, α-PVP, methylone and MDPV	PMMA= 10-1000µM α-PVP=1-300µM Methylone=1-1000µM MDPV=1-1000µM	Male Wistar rats	Postnatal day 0 – 1	Cortical neurons culture	Multi-well microelectrodes arrays	↓ the weighted mean firing rate and weighted mean burst rate

Gerecsei. et al., 2018 (71)	10.3389/fnins.2018.00027	MDPV	10 mg/kg (s.c)	C57Bl/6J mice		Posterior intralaminar complex of the thalamus Medial preoptic nucleus	Open field test Grip Strength Test Force Plate Actometry Pup Retrieval Test Nest Building of pregnant Mothers In Situ Hybridization	↓ birth rate, survival of offspring, and maternal care in the drug-treated animals ↑ locomotor activity of the pups in the MDPV treated group
Gannon, B.M. et al., 2017 (72)	10.1097/FBP.0000000000000315	MDPV and α-PVP	0.032 mg/kg/infusion (i.v)	Male Sprague-Dawley rats	275 - 300 g		Self-administration	both enantiomers of MDPV and α -PVP function as highly effective reinforcers.
Philogene-Khalid, H.L. et al., 2017 (73)	10.1021/acschemneuro.7b00212	R-MEPH and S-MEPH	0.25, 0.50, 2.00 mg/kg/inf (i.v)	Rats			Self-administration Ultrasonic vocalization	↑ break points rats trained to self-administer R-MEPH and greatest rates of 50 kHz ultrasonic vocalization than S-MEPH
Nelson, K.H. et al., 2017 (74)	10.1016/j.pbb.2017.04.003	α-PVP	0.3, 1 and 3 mg/kg (i.p)	Male Sprague-Dawley rats	321 - 445 g		Conditioned place preference Conditioned taste avoidance	α-PVP induced dose-dependent taste avoidance as well as significant increases in time spent on the drug-paired side (no dose-dependent)
Gannon, B.M. et al., 2017 (75)	10.1016/j.drugalcdep.2017.06.031	MDPV	0.03, 0.10, 0.30, and 1.00 mg/ml	Male NIH Swiss mice	20 – 25 g		Two-bottle choice procedures	↑MDPV preference solution when was paired with quinine but no with water ↑ escalate consumption with chronic (10 days) access at 0.30 mg/mL MDPV

							Radiotelemetry Conditioned place preference	↑ CPP at 0.30 mg/mL MDPV with a magnitude similar to the preference observed following intraperitoneal administration of MDPV ↑ locomotor activity at 0.1–1.0 mg/mL MDPV
Lantz, S.M. et al., 2017 (76)	10.1016/j.neuliet.2017.06.059	Phthalimide	10µM-1000µM	Rats		PC12 cells	Cytotoxicity assay	↑death cell after CP exposition CP alters mitochondrial function and DA and 5-HT levels
Luethi, D. et al., 2017 (77)	10.1016/j.tox.2017.06.004	Bupropion, MDPV, mephedrone and naphyrone	0.5-2mM			HepG2 cells HepaRG cells	Cytotoxicity Assay	Bupropion, MDPV, mephedrone and naphyrone are mitochondrial toxicants
Grecco, G.G. et al., 2017 (78)	10.1016/j.taap.2017.05.010	Methylone, butylone and pentylone	20mg/kg (s.c)	Male Sprague-Dawley rats		Blood samples CSF samples	Microdialysis HPLC	↑Cmax and AUC0-∞, and the longest t1/2 In the plasma with pentylone ↑Cmax and AUC0-∞ in the CNS with methylone and butylone
Elmore, J.S. et al., 2017 (79)	10.1038/npp.2016.213	Methylone	3, 6, and 12 mg/kg	Male Sprague-Dawley rats	250 – 300 g	Synaptosomes	Locomotor activity and temperature Monoamine transporter assays In-vivo microdialysis	↑ motor activation at 12mg/kg methylone ↓ core temperature at 3 and 6 mg/kg but showed biphasic effects at 12mg/kg Methylone acted as a fully efficacious substrate-type releaser at DAT, NET, and SERT ↑ brain extracellular dopamine and 5-HT in-vivo
McLaughlin, G. et al., 2017 (80)	10.1002/dta.2053	Mexedrone	Dose-response curve	Male Sprague-Dawley rats	250 – 300 g	Synaptosomes	Monoamine transporter assays	Mexedrone and N-methoxymephedrone showed comparable potency at DAT but the latter compound was more potent at NET and SERT

Schindler, C.W. et al., 2016 (81)	10.1111/bph.13640	MDPV	0.3–3.0 mg/kg	Male Sprague-Dawley rats			Telemetry	S(+) enantiomer appeared to mediate the cardiovascular effects of MDPV
Berquist, M.D. et al., 2016 (82)	10.1016/j.drugalcdep.2016.05.001.	4-MMC and MDPV	0.5 mg/kg (i.p) 0.5, 1.0, or 2.0 mg/kg (i.p)	Male Sprague-Dawley rats			Locomotor activity	Locomotor responses sensitize to MDPV and to certain mixtures of MDPV and 4-MMC following repeated dosing
Schindler, C.W. et al., 2016 (83)	10.1007/s00213-015-4057-0.	MDPV and Methylone	0.03 mg/kg/inj 0.3 or 0.5 mg/kg/inj	Male Sprague-Dawley rats	300 - 400 g		Self-administration Microdialysis Studies	MDPV self-administration was acquired rapidly compare with methylone. MDPV (0.1 and 0.3 mg/kg) increased extracellular dopamine while i.v. methylone (1 and 3 mg/kg) increased extracellular dopamine and 5-HT
Valente, M.J. et al., 2016 (84)	10.1093/toxsci/kfw105	Methylone, MDPV, Pentedrone and 4-MEC	0.05 to 10 or 20 mM	Male Wistar rats	210 - 250 g	HepaRG	Cytotoxic assay	MDPV and Pentedrone were the most cytotoxic. All cathinones triggered significant caspase activation and apoptosis
Colon-Perez, L.M. et al., 2016 (85)	10.1038/npp.2016.40	MDPV	0.3, 1.0, or 3.0 mg/kg	Male Long Evans rats	250 – 300 g	Prefrontal cortex	Functional Magnetic Resonance Imaging	MDPV dose-dependently reduced functional connectivity between frontal cortical and striatal areas Dopamine receptor blockade did not prevent the MDPV-induced decrease in functional connectivity.
Gannon, B.M. et al., 2016 (86)	10.1124/jpet.115.229500	MDPV	0.01, 0.03, 0.10, 0.30, 1, 3, 10 and 30 mg/kg (i.p)	Male NIH Swiss mice	20 – 25g		Drug Discrimination Radiotelemetry	S(+)-MDPV was most potent to fully substitution for the cocaine training dose ↑Locomotion after doses of S(+)-MDPV and racemic MDPV

López-Arnau, R. et al., 2015 (87)	10.1016/j.taap.2015.03.015	Mephedrone	25 mg/kg (s.c)	Male Sprague-Dawley rats adolescent	115 – 130 g 5 weeks	Hippocampus, striatum and frontal cortex	Radioligand binding experiments Western blotting Morris water maze	↓ densities of dopamine and serotonin transporters without microgliosis ↓ expression of tyrosine hydroxylase and tryptophan hydroxylase 2. impairment of the reference memory in the Morris water maze
Kiyatkin, E.A. et al., 2015 (88)	10.1038/npp.2014.191	MDPV and Methylone	1, 3, and 9 mg/kg (s.c) 0.1, 0.3, and 1.0 mg/kg (s.c)	Male Long Evans rats	3 - 4 months		Social interaction	Methylone and MDPV dose-dependently increased brain temperature
Hutchinson, C.V. et al., 2015 (89)	10.1016/j.neuret.2015.03.021	Mephedrone	1 or 10 µM	Flatworm planaria			Conditioned place preference Hypo-locomotion	↑ preference to cocaine at both 1 and 10 µM ↓ locomotion after Mephedrone withdrawal
Lopez-Arnau, R. et al., 2014 (90)	10.1177/0269881114548439	Methylone	4x20 mg/kg (s.c)	Male Sprague-Dawley rats	125 - 175 g 46 weeks	Hippocampus, striatum and frontal cortex	Morris water maze, microglia activation	Hyperthermia, Memory loss
Adam, A. et al., 2014 (91)	10.1016/j.neuro.2014.07.004	MDPV	10 mg/kg	Male and female mice C57	7 d & 16 weeks	Coronal brain slices	Caspase-3	↑ Neuronal apoptosis in young but not adult ↑ locomotion
Den Hollander, B. et al., 2014 (92)	10.1093/toxsci/kfu108	β-keto amphetamine & 4-MMC		Male mice C57BL/6J	8 weeks	SH-SY5Y neuroblastoma cells	Cytotoxicity assay	Cytotoxic
Opacka-Juffry, J. et al., 2014 (27)	10.1016/j.pnpbp.2014.04.009	Mephedrone	0.3-30 µM	Male Wistar rats	8 weeks	Nucleus accumbens	RTI-121 binding, Voltammetry	Displaced RTI-121 and caused reverse transport of DA

Simmler, L.D. et al., 2014 (93)	10.1016/j.neuropharm.2013.11.008	Methedrone, 4-MEC, 3-FMC, pentylone, ethcathinone, buphedrone, pentedrone, and N,N-dimethylcathinone				Human embryonic kidney 293 cells	Monoamine transporter assay	All the cathinones were potent NA uptake inhibitors but different in DA vs 5-HT None of the cathinones bound to rodent trace amine-associated receptor 1
Kaizaki, 2014 (94)		α -PVP	25 mg/kg oral	Male balbC mice	8 weeks		Locomotion Microdialysis	↑ release of DA & locomotion
Marusich, J.A. et al., 2014 (95)	10.1016/j.neuropharm.2014.02.016.	MDPV, α-PVP, α-PBP and α-PPP	0.3–3.0 mg/kg MDPV, 1.0–10.0 mg/kg α-PVP, 1.0–10.0 mg/kg α-PBP, and 3.0–30.0 mg/kg α-PPP	Male Sprague-Dawley rats	300 -400 g	Synaptosomes	Transporter uptake and release assays Locomotor activity	α-PVP is a potent uptake blocker at dopamine and norepinephrine transporters α-PBP and α-PPP are also catecholamine transporter blockers but display reduced potency ↑ locomotor activity with all of them
Watterson, L.R. et al., 2014 (96)	10.1093/ijnp/pyu014	α-PVP and 4-MEC	1, 3, 10, 30, mg/kg 0.1, 0.3, 1, and 3mg/kg	Male Sprague-Dawley rats	250 g		Intracranial self-stimulation in rats	↑ intracranial self-stimulation threshold reductions similar to that of methamphetamine
Bonano, J.S. et al., 2014 (97)	10.1007/s00213-013-3223-5.	MDPV, methylone and mephedrone	0.32, 1 and 3.2 1, 3.2 and 10	Male Sprague-Dawley rats	314 – 387 g		Intracranial self-stimulation	Methcathinone was the most potent compound, and MDPV was the longest acting compound
Gregg, R.A. et al., 2013 (98)	10.1097/FBP.0000000000000006.	Mephedrone	15 mg/kg (i.p)	Male Sprague-Dawley rats	260 – 290 g		Locomotor activity	↑ cocaine-induced locomotor activation by prior MEPH exposure

Gregg, R.A. et al., 2013 (99)	10.1016/j.drugalcdep.2013.06.014.	Mephedrone	15 mg/kg and 30 mg/kg	Male Sprague-Dawley rats	260 –290 g		Sensitization paradigms	↑ repetitive movement by MEPH challenge compared to acute MEPH exposure in both paradigms
Gatch, M.B. et al., 2013 (100)	10.1097/FBP.0b013e328364166d.	MDPV, methylone, mephedrone, naphyrone, flephedrone and butylone	1, 3, 10 or 30 mg/kg 0.3, 1, 3, 10 or 30 mg/kg 3, 10, 30 or 100 mg/kg	Male Swiss–Webster mice	10 weeks		Locomotor activity Discriminative Stimulus Effects	MDPV and naphyrone produced locomotor stimulant effects that lasted much longer than cocaine or methamphetamine All compounds fully substituted for the discriminative stimulus effects of cocaine and methamphetamine
Cameron, K. et al., 2013 (101)	10.1007/s00213-013-2967-2.	MDPV and mephedrone		Female Xenopus laevis		Oocytes	Electrophysiological recordings	mephedrone is a dopamine releasing agent and MDPV behave as a cocaine-like reuptake inhibitor of dopamine.
Baumann, M.H. et al., 2013 (102)	10.1038/npp.2012.204	MDPV	0.1–0.3 mg/kg, (i.v) 0.1–3.0 mg/kg, (s.c)	Male Sprague-Dawley rats Male CB57/BL6 mice	300 - 400 g 25 – 35 g	Synaptosomes Striatum Nucleus accumbens	Uptake and Release assays Fast-Scan Cyclic voltammetry In-vivo microdialysis Telemetry Locomotor Activity	↑ amplitude of the dopamine signal ↑ extracellular concentrations of dopamine in the nucleus accumbens ↑ locomotor activation, tachycardia, and hypertension
den Hollander, B. et al., 2013 (103)	10.1016/j.pbb.2012.10.006	Methylone or mephedrone	30 mg/kg, twice daily for 4 days	Male C57BL/J6 mice Male Wistar rats	8 weeks	Frontal cortex, striatum and hippocampus	Behavioral tests Neurotransmitter and transporters levels	↓ working memory with MEPH ↑ body temperature ↓ 5-HT levels in the frontal cortex, striatum and hippocampus with Methylone

Lopez-Arnau, R. et al., 2013 (104)	10.1016/j.pnpbp.2013.04.007	Methylone	10-30 mg/kg (i.v or oral)	Male Sprague-Dawley rats	225 - 250 g		Locomotion + PK analysis	↑locomotion
Cozzi, N.V. et al., 2013 (105)	10.1016/j.ejphar.2012.11.008	Methcathinone ,2-TFMAP, 3-TFMAP and 4-TFMAP	Methcathinone: 0.3 mg/kg and 1.0 mg/kg 4-TFMAP: 1.0 mg/kg and 3.0 mg/kg	Male Sprague-Dawley rats	300 – 350 g	Human platelets HEK293 cells C6 glioma cells Synaptosomes Accumbens	Monoamine transporter assay Microdialisis Locomotor activity	↑ uptake inhibition and release with 3-TFMAP and 4–TFMAP at SERT compared with methcathinone ↑ 5-HT extracellular level with 4-TFMAP but doesn't affect the locomotor activity
Lisek, R., 2012 (106)	10.1016/j.drugalcdep.2012.04.021.	Mephedrone	3, 5, 10, 30 mg/ kg (i.p) 30 mg/kg (i.p)	Male Sprague-Dawley rats CD-1 mice	225 – 275 g 25 – 30g		Locomotor Activity Conditioned place preference	↑ ambulatory activity in rats and was inhibited by pretreatment with SCH 23390 and enhanced by pretreatment with sulpiride ↑ CPP by MEPH
Meng, H. et al., 2012 (107)	10.1016/j.toxlet.2011.10.010	Mephedrone	0.3-15 mg/kg	Chinese hamster Guinea pigs		Chinese hamster ovary cells Single ventricular myocytes	Patch-clamp Myocyte action potential echocardio	↑HR & ↑BP
Baumann, M.H. et al., 2012 (108)	10.1038/npp.2011.304	Mephedrone and Methylone	0.3 and 1.0 mg/kg 3.0 and 10.0 mg/kg, (s.c) 3 doses	Male Sprague–Dawley rats	300 – 350 g	Synaptosomes	Monoamine transporter assay Microdialysis Locomotion activity	Mephedrone and Methylone similar to MDMA in potency and selectivity for monoamine transporters. ↑ extracellular DA and 5-HT levels after Mephedrone and Methylone i.v administration in accumbens ↑ Locomotor activity and stereotypy after 1mg/kg Mephedrone dose ↑ Locomotor activity after 0.3mg/kg and 1mg/kg Methylone doses.

Table 1. Pre-clinical studies examining cathinone NPS pharmacology. The most recent manuscripts are presented first. DA = dopamine, NA = noradrenaline, DAT = dopamine transporter, NET = noradrenaline transporter, SERT = serotonin transporter, ICSS = intraccranial self-stimulation, USV = ultrasonic vocalisation, CPP = conditioned place preference, MEPH = mephedrone, HR = heart rate, BP = blood pressure.

Table 2. Stimulants

Study	DOI	NPS	Dose/ concentration	Species/ sex	Age	Sample	Assay	Main results
Rickli, A. et al., 2019 (109)	10.1016/j.neuro.2019.02.011	4-MAR, 4,4'-DMAR, and 3,4-DMAR				Human embryonic kidney 293 cells	Monoamine transporter assays	4,4'-DMAR potently inhibited all monoamine transporters. 4-MAR preferentially inhibited the NE and DA transporter 3,4-DMAR only weakly inhibited the NE transporter
Cai, W.T. et al., 2019 (110	10.1016/j.neuint.2019.104487	Methiopropamine	5 mg/kg (i.p)	Rats		Nucleus accumbens		↑number of total spine density
Maier, J. et al., 2018 (111)	10.1016/j.neuropharm.2018.06.018	4,4'-DMAR	Dose-response curve	Human/ rat		Human embryonic kidney 293 cells Human striatal tissue Rat pheochromocytoma cells (rPC12)	Uptake inhibition assays Transporter release assays Receptor and transporter binding and activation assays	↓ uptake mediated by human DAT, NET or SERT Release assays identified 4,4'-DMAR as a substrate type releaser, capable of inducing transporter-mediated reverse transport via DAT, NET and SERT ↓ rat and human isoforms of VMAT2 at a potency like MDMA
Davidson, C. et al., 2018 (112)	10.3389/fpsyt.2018.00149	3,4-CTMP and ethylphenidate	1, 10 and 100 nM/ 1 and 10 µM	Male Wistar rats	8 weeks	Nucleus accumbens and stria terminalis	Fast cyclic voltammetry (DA and NA)	↑ evoked dopamine and noradrenaline efflux with Methylphenidate (10 µM) ↑ evoked dopamine and noradrenaline efflux with 3,4-CTMP (0.1 and 1 µM) ↑ evoked dopamine and noradrenaline efflux with Ethylphenidate (1 µM)

Luethi, D. et al., 2018 (113)	10.1016/j.neuropharm.2017.08.020	N-benzylethylphenidate, 3,4-dichloroethylphenidate, 3,4-dichloromethylphenidate, ethylnaphthidate, ethylphenidate, 4-fluoromethylphenidate, isopropylphenidate, 4-methylmethylphenidate, methylmorphenate, and propylphenidate				Human embryonic kidney 293 cells	Monoamine transporter assays	↑ inhibition NAT and DAT with all the drugs No cytotoxicity was observed after drug treatment at assay concentrations.
McLaughlin,G. et al., 2018 (114)	10.1002/dta.2396	4-MPM/3-MPM	Dose-response curve	Male Sprague-Dawley rats	250 – 300 g	Caudate (for DAT assays) or whole brain minus cerebellum and caudate (for NET and SERT assays)	Monoamine transporter assays	2-MPM and 3-MPM will exhibit stimulant properties like the parent compound phenmetrazine, whereas 4-MPM may display entactogen properties more similar to MDMA.
Zwartsen, A. et al., 2018 (115)	10.1016/j.neuro.2018.03.007	BZP and TFMPP	BZP=1-1000µM TFMPP=1-1000µM	Male Wistar rats	Postnatal day 0 – 1	Cortical neurons culture	Multi-well microelectrodes arrays	↓ the weighted mean firing rate and weighted mean burst rate
Mayer, F.P. et al., 2018 (116)	10.1016/j.neuropharm.2017.10.006	3-FPM, 2-FPM and 4-FPM	Dose-response curve	Male Sprague-		Human embryonic	Monoamine transporter assays	2-, 3- and 4-FPM inhibit uptake mediated by DAT and NET

				Dawley rats		kidney 293 cells Synaptosomes	Patch clamp	All FPM raised concentration-dependent release of monoamines from rat brain synaptosomes
Sahai, M.A. et al., 2017 (37)	10.1016/j.pnpbp.2016.11.004	5-MAPB	1,3,10 and 30 µM	Male Wistar rats	8 weeks	Striatum Accumbens	Radioligand binding Fast cyclic voltammetry	5-MAPB reduces the rate of dopamine reuptake 5-MAPB binds to the DAT and displace RTI-121 as DAT ligand
Shimshoni, J.A. et al., 2017 (117)	10.1016/j.taap.2017.01.018	MEAI	10 and 30 mg/kg	Male Sprague-Dawley rats		Striatum primary neurons Human primary hepatocytes	Safety profile Cytotoxic assay	Good safety profile in rats at 10 and 30mg/kg Cytotoxic effect at 500 and 1000mg/L concentrations
McLaughlin, G. et al., 2017 (118)	10.1002/dta.2167	4F-MPH	Dose-response curve	Male Sprague-Dawley rats	250 – 300 g	Synaptosomes	Monoamine transporter assays	↑ potencies determined for blockage of dopamine uptake and norepinephrine uptake in (±)-threo isomer ↓ potent at the dopamine transporter and norepinephrine transporter in MPH
Yoon, H.S. et al., 2016 (119)	10.1016/j.bbr.2016.05.060	Methiopropamine	i.p. 0.2, 1.0, or 5.0 mg/kg	Male Sprague-Dawley rats	220 – 250 g		Locomotor activity	↑ sensitized locomotor activity the group that was pre-exposed at 5mg/kg of MPA MPA-induced locomotor sensitization was inhibited by a pre-injection of a dopamine D2 receptor antagonist
Marusich, J.A. et al., 2016 (120)	10.1016/j.neuropharm.2015.09.004	5-IT and 6-IT	1.0–10.0 mg/kg	Male Sprague-Dawley rats	300 - 400 g	Synaptosomes	Locomotor activity Monoamine transporter assays	5-IT displayed greater potency for release at DAT over SERT, while 6-IT displayed greater potency for release at SERT over DAT 5-IT produced locomotor stimulation
Simmler, L.D. et al., 2016 (121)	10.1124/jpet.115.229765	101 compounds Amphetamines Phenethylamines	10 pM to 10 µM			Human embryonic	Radioligand binding Assay	Species differences in activity at TAAR1 among the highly active ligands, with a rank order of rat > mouse > human

		Aminoindanes, cathinones, ephedrines, tryptamines, piperazines andpipradrols				kidney 293 cells Rat and mouse TAAR1	Functional TAAR1 Activity	
Asaoka, N. at al., 2016 (122)		5F-ADB	1µM			Midbrain dopaminergic neurons	Electrophysiol ogical recordings	5F-ADB significantly increase the spontaneous firing rate in dopaminergic but nor in serotoninergic neuron
Persona, K. et al., 2016 (123)	10.1007/s12640-016-9604-x	N-Benzylpiperazine	0.1, 0.3, 1, 3, 10, 30, 100, 300, and 1000 µg/ml 10, 30, 100, and 300 µg/ml			Human glioblastoma cells (LN-18)	Cytotoxicity assay Real-Time PCR Gene Expression analysis	↑ LDH levels ↑ mitochondrial membrane potential, ↓ ATP and ↑ ROS production, ↑ levels of DNA damage marker (8-OHdG) and activation of caspases: -3 and -9.
Arbo, M.D. et al., 2016 (124)	10.1007/s00204-016-1665-3	1benzylpiperazine, 1 (3,4methylenedioxy benzyl) piperazine, 1-(3-trifluoromethylphenyl) piperazine and 1-(4-methoxyphenyl) piperazine	625, 210 and 0.5 µM for BZP 35, 12 and 0.5 µM for TFMPP 522, 175 and 0.5 µM for MeOPP 467, 160 and 0.5 µM for MDBP	Male Wistar rats	300 – 400 g	Hepatocytes	Cytotoxicity assay Gene array	Key enzymes of cholesterol biosynthesis, glycoprotein transmembrane nmb and fatty acid desaturase 1 were up-regulated by all four piperazine drugs The betaine-homocysteine-S-methyltransferase 2 were down-regulated all four piperazine derivatives
Arbo, M.D. et al., 2016 (125)	10.1002/jat.3153	N-benzylpiperazine, 1-(3-trifluoromethylphenyl) piperazine, 1-	500 or 1000 µM BZP 5, 50 or 100 µM TFMPP			SH-SY5Y cell	Cytotoxic assay	1-(3-trifluoromethylphenyl) piperazine was the most cytotoxic

		(4-methoxyphenyl)piperazine and 1-(3,4-Methylenedioxybenzyl)piperazine	250 or 500 µM MeOPP or MDBP					
Rubio, M. et al., 2015 (126)	10.1016/j.neulet.2015.01.075	4-Methylamphetamine (4-MA)	s.c. 2.5, 5 and 10 mg/Kg	Male Sprague-Dawley rats	125 – 175 g		Locomotor activity Radiotelemetry	↑dose-dependent manner, locomotor activity dose-dependent hypothermic response to 4-MA
Rickli, A. et al., 2015 (127)	10.1016/j.euroneuro.2014.12.012	Para-halogenated amphetamines and pyrovalerone cathinones				Human embryonic kidney 293 cells	Monoamine transporters assays	↑ 5-HT properties in 4-methyl, 4-ethyl, and 4-bromo groups 3,4-methylenedioxypyrovalerone, pyrovalerone, α-pyrrolidinovalerophenone, 3,4-methylenedioxy-α-pyrrolidinopropiophenone, and 3,4-methylenedioxy-α-pyrrolidinobutiophenone potently inhibited the NET and DAT but not the SERT
McLaughlin, G. et al., 2015 (128)	10.1002/dta.1732	MDMAR & DMAR	1 nM – 10 µM	Male Sprague-Dawley rats	250 - 300 g	Synaptosomes	Synaptosomal uptake/release	More efficacious than MDMA
Arbo, M.D. et al., 2014 (129)	10.1016/j.toxlet.2014.06.031	N-benzylpiperazine, 1-(3-trifluoromethylphenyl)piperazine, 1-(4-methoxyphenyl)piperazine and 1-(3,4-methylenedioxybenzyl)piperazine				H9c2 rat cardiac cell line	Cytotoxic assay	TFMPP seems to be the most potent cytotoxic compound

Brandt, S.D. et al., 2014 (130)	10.1002/dta.1668	4,4'-DMAR	0.3nM – 10 µM	Male Sprague-Dawley rats	250 - 300 g	Synaptosomes	Monoamine transporters assays	↑ release of DA, NA, 5-HT. more potent than AMPH at 5-HT
Dawson, P. et al., 2014 (36)	10.1016/j.pnpbp.2013.08.013	5-APB	0.3 - 30 µM	Male Sprague-Dawley rats	8 weeks	Prefrontal cortex, nucleus accumbens and caudate–putamen	Binding and voltammetry in slices. Fundus and aorta preps	Displaced both DAT and 5-HT2 ligands and ↑ DA efflux. ↑ contraction in fundus and aorta
Davidson, C. et al., 2012 (47)	10.1177/0269881111430733	Desoxypipradrol	1 - 10 µM	Male Sprague-Dawley rats	8 weeks	Nucleus accumbens	Voltammetry in slices	Bigger increase in DA vs cocaine

Table 2. Pre-clinical studies examining stimulant NPS pharmacology. The most recent manuscripts are presented first. DA = dopamine, NA = noradrenaline, DAT = dopamine transporter, NET = noradrenaline transporter, SERT = serotonin transporter.

Table 3. synthetic cannabinoids

Study	DOI	NPS	Dose/conc	Species/sex	Age	Sample	Assay	Main results
Bilel, S. et al., 2019 (131)	10.3389/fnins.2019.01163	AKB48	0.25 mg/kg to 3 mg/kg	Male rats		Nucleus accumbens	Pharmacological and behavioural effects	↑ DA release in the nucleus accumbens shell at 0.25 mg/kg ↓ startle/pre-pulse inhibition response at 3mg/kg ↑ hypothermia, analgesia, and catalepsy at 3mg/kg ↑impaired place preference and hypolocomotion at 0.5mg/kg
Banister, S.D. et al., 2019 (132)	10.1002/dta.2491	SCRAs 5F-CUMYL-PICA, 5F-CUMYL-PINACA and 5F-CUMYL-P7AICA	0.1, 0.3, 1, and 3 mg/kg (i.p)	Male C57BL/6J mice	21.2 – 29 g	Human embryonic kidney 293 cells AtT20 adenocarcinoma cells	Binding assay Functional activity assay In vivo pharmacological assessment	All compounds were potent CB1 agonists 5F-CUMYL-P7AICA induced hypothermia
Kim, S. et al., 2019 (133)	10.3390/molecules24163000	AKB48	0.1 to 100 µM			Human liver microsomes LLC-PK1-MDR1 LLC-PK1-mock cells HEK293 cells HEK293-mock cells	Cytochrome P450s assays	↑inhibition of CYP3A4 and UGT1A9 activities
Kevin, R.C. et al., 2019 (134)	10.3389/fphar.2019.00595	CUMYL-4CN-BINACA	0.03, 0.1, 0.3, and 1 mg/kg (i.v)	Male C57BL/6J mice		Mouse AtT20FlpIn neuroblastoma cells Human embryonic	Radioligand binding assay Receptor functional assay Biotelemetry	Potent CB1 receptor agonist ↑ pro-convulsant effects at 0.3 mg/kg

						kidney 293 cells	Locomotor activity	
Domoto, M. et al., 2018 (135)	10.1007/s00213-018-4933-5	5F-AMB	300 nM	Male and female C57BL/6J mice	4−6 weeks	Medial Prefrontal cortex	Patch-clamp	↓ excitatory and inhibitory transmission in mPFC L5 pyramidal neurons via the activation of CB1 receptors located in presynaptic terminals
De Luca, M.A. et al., 2016 (136)	10.1016/j.neuropharm.2015.11.017	BB-22, 5F-PB-22, 5F-AKB-48 and STS-135	BB-22: 0.003-0.1 mg/kg 5F-PB-22: 0.01 mg/kg 5F-AKB-48: 0.1 mg/kg STS-135: 0.15 mg/kg	Male Sprague-Dawley rats C57BL/J6 and CB1knockout (KO) mice	200 – 300 g 17 – 20 g	Nucleus Accumbens Medial Prefrontal cortex	Binding assay In vivo microdialysis	5F-AKB-48 and STS-135 had higher Ki for CB1 binding. ↑ DA in the accumbens shell with all the compounds
Yun, J. et al., 2016 (137)	10.1039/c6tx00259e	JWH-030	0.1, 1, 10, or 100 µM 0.001–2500 µM 30 µM i.v. 0.5 mg/kg	Male Sprague-Dawley rats Male New Zealand white rabbits	7 weeks 2890–3380g	H9c2 cells Rabbit Purkinje fibers	Cytotoxic assay Patch clamp Action potential duration (APD) Electrocardiogram	JWH-030 was more cytotoxic than JWH-210, JWH-250 and RCS4 JWH-030 to block the hERG channel JWH-030 significantly reduced the APD at 90% repolarization JWH-030 prolonged the QT interval in rats
Tomiyama, K. 2014 (138)	10.1016/j.taap.2013.10.028	CP-55,940, CP-47,497, CP-47,497-C8, HU-210, JWH-018, JWH-210, AM-2201, and MAM-2201	1-30 µM for 0.5-3 h	ICR mouse	15 days gestation	Primary neuronal cell culture	Forebrain cell cultures for caspase-3	Apoptosis

Table 3. Pre-clinical studies examining synthetic cannabinoid receptor agonist NPS pharmacology. The most recent manuscripts are presented first. DA = dopamine, NA = noradrenaline, DAT = dopamine transporter, NET = noradrenaline transporter, SERT = serotonin transporter.

Table 4. Other NPS

Study	DOI	NPS	Dose/conc	Species/sex	Age	Sample	Assay	Main results
Luethi, D. et al., 2019 (139)	10.1016/j.ejphar.2019.05.014	2C-BI derivatives				Human cells	Monoamine transporter assays	2C-BI-8 and 2C-BI-12 activated serotonin 5-HT2A and 5-HT2B receptors at submicromolar concentrations
Wallach, J. et al., 2019 (140)	10.1016/j.ejphar.2019.172427	Fluorolintane and its five aryl-fluorine-substituted isomers		Rats		Hippocampal slices	Monoamine transporter assays Field-recording electrophysiology Behavioural test	↑affinity for NMDA receptors ↓ long-term potentiation ↓NMDA receptor-induced field excitatory postsynaptic potentials ↓ PPI
Costa, G. et al., 2019 (141)	10.1016/j.neuropharm.2018.10.031	Methoxetamine	0.1-0.5 mg/kg, i.p., × 5 days	Rats			Ultrasonic vocalizations Behavioural test	Pre-treatment with MXE impair alterations in the elevated plus maze, marble burying and novel object ↑ neurotoxicity
Yoon, K.S. et al., 2019 (142)	10.1007/s12012-018-9489-4	Methoxetamine	0.1–500 µM	Mice		H9c2 cells	Cytotoxic assays	↓ cell viability and PAK-1 mRNA levels at 10 µM ↓beating rate of primary cardiomyocytes at 100 µM
Halberstadt,A. L. et al., 2019 (143)	10.1016/j.neuropharm.2018.10.037	DOB, 2C-B and benzodifuranyl and tetrahydrobenzodifuranyl analogs		C57BL/6J mice			Head twitch response (HTR) assay	DOB and 2C-B induced the HTR ↑potency of DOB-DFLY and 2C-B-DFLY than DOB and 2C-B 2C-I-FLY, 2C-E-FLY and 2C-EF-FLY active in the HTR assay but had low potency
Herian, M. et al., 2019 (144)	10.1007/s12640-019-00033-x	25I-NBOMe	0.3, 1, 3, and 10 mg/kg	Male Wistar-Han rats	280 – 350 g	Frontal cortex	Microdialysis	Inverted U-shaped dose-response curve on extracellular DA and 5-HT levels

								U-shaped dose-response curve on GLU levels
Zwartsen, A. et al., 2018 (71)	10.1016/j.neuro.2018.03.007	2C-B, 25B-NBOMe	2C-B=1-300µM 25B-NBOMe=0.01-30µM	Male Wistar rats	Postnatal day 0 – 1	Cortical neurons culture	Multi-well microelectrodes arrays	↓ the weighted mean firing rate and weighted mean burst rate
Shintani-Ishida, K. et al., 2018 (145)	10.1111/1556-4029.13583	25B-NBOMe	i.p. 0.5 mg/kg	Male Sprague-Dawley rats	8 weeks	Blood samples Lung, heart, kidney or brain tissue homogenate	HPLC	↑ 25B-NBOMe concentration in blood samples after 6 hours 25B-NBOMe accumulated primarily in the lung
Cha, H.J. et al.,2018 (146)	10.1016/j.neulet.2018.04.009	4-chloro-2,5-dimethoxyamphetamine and AH-7921	DOC: 0.1, 0.3, and 0.5 mg/kg AH-7921: 0.1, 0.3, and 1 mg/kg	Male Sprague-Dawley rats C57BL/6 mice	180 – 220 g 15 – 20 g		Conditioned place preference Self-administration	↑ preference with both drugs at 0.3 mg/kg ↑ number of responses to the active lever in the self-administration test at 0.01 mg/kg
Luethi, D. et al., 2018 (7)	10.1016/j.ejphar.2017.12.012	Diclofensine, diphenidine, and methoxphenidine				Human embryonic kidney 293 cells	Monoamine transporter assays	Diclofensine bound to adrenergic, dopamine, serotonin, and trace amine-associated receptors. Diphenidine bound to adrenergic α_{1A} and α_{2A} receptors and serotonin 5-hydroxytryptamine 1A (5-HT_{1A}) and 5-HT_{2A} receptors. Methoxphenidine bound to adrenergic α2A and serotonin 5-HT2A and 5-HT2C receptors
Hondebrink, L. et al., 2017 (52)	10.1016/j.neuropharm.2017.04.035	Methoxetamine	0.1 M	Male Wistar rats	Postnatal day 1	Rat primary cortical cells Human SH-SY5Y cells	Cell intracellular calcium imaging	↑ the glutamate-evoked increase in [Ca2+] in rat primary cortical cells with 10 µM methoxetamine

						Human embryonic kidney 293 cells	Multi-well microelectrodes arrays Monoamine transporter assays	↓ the K+- and acetylcholine-evoked increase in [Ca2+]I in human SH-SY5Y cells with 10 µM methoxetamine ↓ spontaneous neural activity between 10-100µM methoxetamine ↓ uptake via monoamine transporters (DAT, NET and SERT)
Kang, H. et al., 2017 (147)	10.1016/j.neuropharm.2016.08.004	Ephenidine	Dose-response curve 1 and 10 µM	Male rats (Wistar and Sprague Dawley)	3 – 10 weeks	Whole rat brain CA1 of rat hippocampal slices Hippocampal pyramidal cells	Receptor binding assays Extracellular recording of field excitatory postsynaptic potentials Patch clamp	Ephenidine acts at the PCP site of the NMDA receptor and has lower affinity for the dopamine and noradrenaline transporters ↑ inhibition of the NMDA receptor mediated fEPSP at 10 µM 10 µM blocked NMDA receptor-mediated EPSCs
Rickli, A. et al., 2016 (148)	10.1016/j.euroneuro.2016.05.001	DiPT, 4-OH-DiPT, 4-OH-MET, 5-MeO-AMT, and 5-MeO-MiPT Lysergic acid diethylamide, psilocin, N,N-dimethyltryptamine and mescaline					Monoamine transporter assay	↓ binding 5-HT2A with all the tryptamines and psilocin and DMT compared with LSD DMT, DiPT, 4-OH-DiPT, and 4-OH-MET, interacted partially with the norepinephrine transporter LSD but not the tryptamines interacted with adrenergic and dopaminergic receptors
Wallach, J. et al., 2016 (149)	10.1371/journal.pone.0157021	DPH, 2-MXP and 3- and 4-MeO- isomers and 2-Cl-	1uM and 10uM 1.25,2.5,5 and 10mg/kg (s.c)	Male Wistar rats Male Sprague–Dawley rats	9 - 10 weeks 250 – 275 g	Whole brain Hippocampal slices	Binding studies Monoamine Reuptake Inhibition Assays In-vitro Field Excitatory Postsynaptic potential	DPH and 2-MXP, were found to be relatively selective NMDAR antagonists and inhibited NMDAR mediated field EPSPs

		diphenidine (2-Cl-DPH)					Pre-pulse inhibition	DPH and 2-MXP significantly inhibited PPI
Rickli, A. et al., 2015 (34)	10.1016/j.neuropharm.2015.08.034	2C drugs				Human cells	Monoamine transporter assay	NBOMe drugs were very potent 5-HT2A receptor agonists 2C drugs increased the binding affinity at serotonergic 5-HT2A, 5-HT2C, adrenergic α1, dopaminergic D1-3, and histaminergic H1 receptors and monoamine transporters but reduced binding to 5-HT1A receptors and TAAR1
Blough, B.E. et al., 2014 (150)	10.1007/s00213-014-3557-7	Tryptamines (21 compounds)		Rats		Synaptosomes	Monoamine transporter assay	All tryptamines were 5-HT2A agonists. N-ethyltryptamine was the greatest 5-HT releaser and 5-MeO-MIPT was the weakest 5-HT uptake inhibitor.
Dargan, P.I. et al., 2014 (30)	10.3109/15563650.2014.892605	Methoxetamine	i.p 30 mg/kg x 3 months	Mice	2-5 months	Bladder and kidneys	CD4 & Sirius red staining	Bladder and renal toxicity
Paulke, A. et al., 2013 (151)	10.1016/j.jep.2013.04.044	Argyreia nervosa and LSA					In silico and radioligand assays	Lower affinity than LSD but clear affinity at 5-HT1A, 5-HT2, and α2
Compton, D.M. et al., 2011 (152)	10.1016/j.physbeh.2011.01.021	5-MeO-DIPT	6 x 5 or 20 mg/kg	Male Long Evans rats	35 or 48 days		Variety of behavioural tests	Cognitive deficits

Table 4. Pre-clinical studies examining ‘other’ NPS pharmacology. The most recent manuscripts are presented first. DA = dopamine, NA = noradrenaline, DAT = dopamine transporter, NET = noradrenaline transporter, SERT = serotonin transporter, PPI = pre-pulse inhibition.